Trauma Induced Secondary Cardiac Injury

Clinical manifestations and underlying mechanisms

Sriveena Naganathar

Centre For Trauma & Neurosciences
The Blizard Institute
Queen Mary, University of London

This thesis has been submitted in partial fulfillment of the requirements of the Degree of Doctor of Philosophy

2017

Supervisors

Professor Karim Brohi, FRCS FRCA

Professor of Trauma Sciences
Blizard Institute of Cell and Molecular Science
Queen Mary University of London

Professsor Joanne Martin MA MBBS PhD FRCPath

Professor of Pathology
Centre for Genomics and Child Health
Queen Mary University of London

Abstract

Since 1933, studies have explored the concept of trauma induced secondary cardiac injury (TISCI), yet till 2012, it had not been defined as the incidence of cardiac events and rise in cardiac biomarkers following traumatic injury. Despite, improvements in early outcomes, trauma patients have reduced long-term mortality with cardiac disease being the major contributor. Although many putative mechanisms have been suggested for TISCI, the underpinning pathophysiology still remains unclear.

In this thesis, a prospective study of 290 critically injured patients identifies a 13% incidence of adverse cardiac events (ACE) with consistently raised serum h-FABP levels in these patients. H-FABP was found to be a good predictor of ACE through ROC analysis and a h-FABP of 16.8 ng/ml used to define trauma induced secondary cardiac injury (TISCI). TISCI was associated with longer hospital stay and higher mortality. Patients who developed ACE had higher plasma levels of adrenaline and noradrenaline with a correlating increase in plasma h-FABP. On multivariate analysis, hypertension was the only independent risk factors for ACE.

The increase in serum cardiac biomarkers was reflected by an increase in serum h-FABP in our group's trauma hemorrhage murine models. The hearts of these models were used in the experiments that form the last experimental chapter of this thesis. Protein expression studies confirm this increase in serum h-FABP by

evidence of concurrent leaching in the cardiac tissue, along with Troponin I. Myocardial injury was evident on electron microscopy with evidence of interstitial and organelle oedema, myofibrillar degeneration, nuclear condensation and changes in mitochondrial morphology. Immunohistochemistry and western blotting protein studies demonstrate the translocation of the mitochondrial death-related protein AIF to the cytosol and nucleus, where it becomes its active pro-apoptotic form.

This thesis propositions the utility of the cardiac biomarker h-FABP in predicting ACE and outcomes in critically injured patients. Although increasing serum noradrenaline and adrenaline levels are associated with higher incidence of ACE and biochemical evidence of cardiac injury with rising h-FABP levels, multivariate analysis negates their value as independent predictors of ACE. Leaching out of the proteins h-FABP and Troponin I in the murine cardiac tissue confirmed the value of serum measurements of these proteins as markers of cardiac injury. This was associated with widespread ultrastructural myocardial damage in the TH mice with changes in mitochondrial morphology. The mitochondrial damage seen is associated with the translocation of the mitochondrial death-related protein AIF to the cytosol and the nucleus where I propose its canonical signaling leading to nuclear degradation and cell death is the driver of cardiac dysfunction.

Statement of Originality

I, Sriveena Naganathar, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

I accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis.

I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university.

Signature:

Date: 02nd October 2017

Publications and Presentations

Published Work

Admission biomarkers of trauma induced secondary cardiac injury predict adverse cardiac events and are associated with plasma catecholamine levels.

Naganathar S, De'Ath HD, Wall J, Brohi K.

Journal of Trauma and Acute Care Surgery. 2015 July; 79 (1): 71-7

Oral Presentation

Adverse Cardiac Events and Catecholamines In Injured Patients.

Raymond H. Alexander Presidents Paper Prize competition Finalist 2015

Eastern Association for the Surgery of Trauma 28th Annual Scientific Assembly. Florida, USA

Trauma Induced Secondary Cardiac Injury

TRAUMA *Talks Series 2015*

Royal London Hospital, United Kingdom

Adverse Cardiac Events and Catecholamines In Injured Patients.

Graduate Study Day Postdoctoral Presentations

Blizard Institute, Queen Mary University Of London, United Kingdom

Acknowledgements

First and Foremost, I would like to thank Professor Karim Brohi for the privilege of pursuing independent research, his mentorship and patience.

I am very grateful to Professor Joanne Martin for her expert guidance and resourcefulness. I would also like to express my gratitude to Dr Henry De'Ath for the conception of the idea of TISCI and his support throughout my PhD and Dr Johanna Wall for her collaborative spirit and her expertise with the animal models.

I am indebted to the patients and their family who magnanimously give us permission to conduct our research in spite of their difficult circumstances. I cannot thank you enough.

My time at the Centre for Trauma Sciences has been truly enriching due to the wonderful people in it, who were not only great colleagues and inspiring researchers but also dear friends. I would like to particularly thank Dr Scarlett Gillespie & Miss Emma Mallas for their friendship, support and kindness. None of this would have been possible without the diligence of my fellow comrades, the research fellows, past and current. A special thanks to Dr Hemanth Tummala for his tutelage, patience and his constant generosity with his time. Thank you also to Dr Ping Yip and Dr Jordi Lopez-Tremolada for his expert advice and guidance.

I am eternally grateful for my friends, my pillars of strength, for their counsel, their grandiose faith in me and for sharing this experience with me every step of the way. Most importantly, I would like to thank my family- my grandmother, my mother and my father who sacrificed their dreams so I could live mine. I cannot express enough gratitude to my brother, sister, sister-in-law and brother-in-law and for their love, support and encouragement. And finally, I would like to thank my nieces, Aalaya and Yaara for inspiring me.

I dedicate this thesis to all the women in my life, thank you for your untiring and selfless nurturing.

Table of Contents

List of figures

List of Tables

List of Abbreviations

A&E	Accident and Emergency
AA	Atrial Arrhythmias
ACE	Adverse Cardiac Events
ACEi	Angiotensin-converting-enzyme inhibitor
ACS	Acute Coronary Syndrome
AF	Atrial Fibrillation
AIF-1	Apoptosis Inducing Factor 1
AIS	Abbreviated Injury Scale
AMI	Acute Myocardial Infarction
ATLS	Advanced Trauma Life Support
ATP	Adenosine Triphosphate
AUC	Area Under Curve
BCI	Blunt Cardiac Injury
BNP	Brain Natriuretic Peptide
CCU	Critical Care Unit
CK	Creatine Kinase
COPD	Chronic Obstructive Pulmonary Disease
cTnI	Cardiac Troponin I
CVA	Cerebral Vascular Accident
DALY	Disability Adjusted Years
DAPI	4', 6 - Diamidino-2-Phenylindole
ddH2O	Double Distilled Water
DNA	Deoxyribonucleic Acid
ECG	Electrocardiogram
ED	Emergency Department
ED vol	End Diasystolic Volume
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ES vol	End Systolic Volume
H-FABP	Heart-Specific Fatty Acid Binding Protein
H&E	Hematoxylin and Eosin
HCL	Hydrochloric Acid
ICU	Intensive Care Unit
IHD	Ischaemic Heart Disease
IL	Interleukin
ISS	Injury Severity Score
IVSDd	Interventricular Septum Diastolic Diameter
LAD	Left Axis Deviation
LAR	Legally Authorised Representative
LDH	Lactacte Dehydrogenase
LTS	London Trauma System
LV	Left Ventricular

LVEDD	Left Ventricular End Diastolic Diameter
LVESD	Left Ventricular End Systolic Diameter
LVID	Left ventricular Internal Diameter
LVOT	Left Ventricular Outflow Tract
LVSV	Left Ventricular Stroke Volume
MAP	Mean Arterial Blood Pressure
ml	Milliltres
MLS	Mitochondrial Localisation Sequence
mM	Millimolar
MODS	Multiple Organ Dysfunction
mol/l	Moles per litre
MRC	Mitochondrial Respiratory Complex
MRI	Magnetic Resonance Imaging
ng/ml	Nanogram per milliliter
nm	Nanometre
nt-Pro BNP	N-terminal (NT)-pro hormone Brain natriuretic peptide
OD	Optical density
PBS	Phosphate buffered saline
PE	Pulmonary emboli
PLAR	Professional Legally Appointed Representative
PVD	Peripheral vascular disease
PVDF	Polyvinylidene difluoride
RAD	Right Axis Deviation
RBBB	Right Bundle Branch Block
RIPA	Radioimmunoprecipitation assay
ROC curve	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
rpm	Revolution Per Minute
RT	Room Temperature
RTA	Road Traffic Accident
SAH	Subarachnoid Haemorrhage
SBP	Systolic Blood Pressure
SDS	Sodium Dodecyl Sulfate
SIRS	Systemic Inflammatory Response Syndrome
TACID	Trauma Associated Cardiac Injury and Death
TBI	Trauma Brain Injury
TH	Trauma Haemorrhage
TISCI	Trauma-Induced Secondary Cardiac Injury
TMB	3,3'5,5' - Tetramethylbenzidine
TNF	Tumour Necrosis Factor
TNFR	Tumour Necrosis Factor Receptor
UTI	Urinary Tract Infection
VAP	Ventilator Acquired Pneumonia
VTI	Velocity Time Integral
µl	Microlitre

Chapter 1

Introduction

1.1 Burden of Disease

Injury is defined as the transfer of energy leading to tissue damage. This still remains a pertinent global health issue, contributing to 9% of global mortality, which translates to 5 million deaths every year (1). It is the leading cause of death in young people in the UK, with a total of 48000 cases of major trauma each year resulting in 19500 deaths(2).

Road traffic accidents (RTA) alone account for 24% of trauma deaths and are predicted to be the fifth leading cause of mortality by 2030. About 1.25 million deaths a year occur due to RTA injuries, with 20- 50 million more suffering from non-fatal injuries (3). It is estimated that 3500 lives are lost each day secondary to RTAs. Unsurprisingly, there is a large incongruence in mortality rates between economic regions worldwide. Despite owning only 56% of registered vehicles worldwide, 90% deaths due to RTA occur in low/middle income countries due to the lack of safety law enforcement (3).

Although globally we have seen a 17% decrease in violence, in middle/low income countries of the Americas there has been a 5% increase with incidence rates that are four fold higher than the global average (3). Trauma also contributes to a greater disease burden in this population owing to high rates of violence against women and homicide, with 75% attributed to gun crime (3).

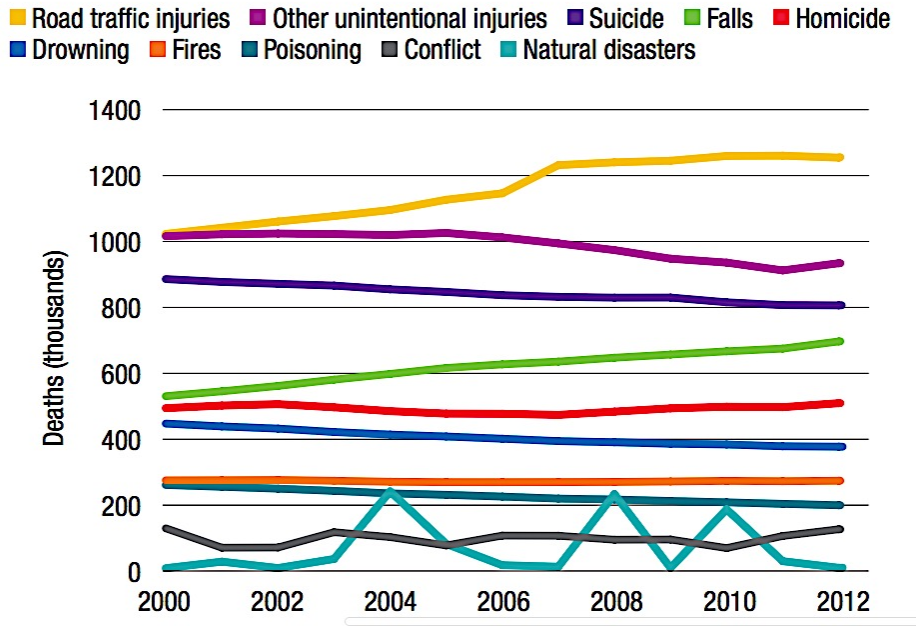


Figure 1.1 Global trends of deaths attributable to injuries from 2000-2012 *(3)*

It is estimated that for every death due to traumatic injury, two people are left with life changing injuries. In addition to reduced physical function, patients are left with chronic pain, cognitive issues and psychological distress. Globally, trauma leads to an astonishing loss of 12% of total DALY (Disability adjusted years) due to all causes (4,5).

Although trauma only contributes to 0.2% of A&E workload, its immediate treatment costs more than £300 million with annual economic output estimated to be in between £3.3-3.7 billion (2). Worldwide injuries lead to a loss of 3% of the global gross domestic product (4).

Despite the global improvements in outcome, trauma still remains a significant burden on health and socioeconomic outcomes in the UK and worldwide.

1.2 Causes of Death in Trauma

The temporal pattern of death in trauma has historically been described as trimodal. More recent studies have noted shifts due to changes in pre-hospital care and resuscitation practices. The majority of deaths associated with trauma occur in the acute phase, within hours of injury (6,7). The leading cause of death in trauma patients is catastrophic brain injuries and then secondly major haemorrhage (7,8). The advent of the London Trauma System (LTS) and its introduction of triaging patients to specialised Level I trauma centres has led to significant improvements in patient outcomes in a short period of time (9). In just over 3 years, mortality within the first 72 hours reduced from 14% to 7%. The establishment of the LTS was preceded by the development of major haemorrhage protocols involving optimisation of transfusion practices, the use of tranexamic acid and the promotion of damage control resuscitation and surgery all being central to the reduction in mortality and improvement in trauma care (9).

On hospital admission, patients who survive the acute post injury phase, have a greater risk of developing multiple organ dysfunction (MODs) during their hospital admission. Improvements in critical care protocols have significantly reduced the incidence of MODs over time, however mortality remains unchanged (7,10). In patients who develop MODs, although deaths primarily due to cardiac dysfunction have declined, it still contributes to MODs in 5% of the non-survivors(10,11). In a large study of the German Trauma registry, Frohlich 2013 showed that circulatory

failure occurred in in 33% patients(12,13). A single centre study by the same group, also showed that trauma patients remain at risk of higher long-term mortality rate when compared to gender and age matched non-injured group (10-14). Cardiovascular disease was the highest cause of mortality, contributing to 22% of deaths in trauma patients post discharge (14).

1.3 Preclinical Evidence for Cardiac Dysfunction in Trauma

Most of the evidence for the existence of myocardial dysfunction following trauma haemorrhage comes from rodent models. Professor Chaudry's group has significantly contributed to this field since 1993. They have focused on depression in myocardial function and explored the mechanistic contribution of sex hormones and inflammatory proteins. Their model uses rats that are anaesthetised with methoxyfluorane and subjected to a 5 cm midline laparotomy. Vascular access lines are placed in the carotid artery, femoral artery and femoral vein and the rodents are bled to a mean arterial pressure of 40mmHg. Initial resuscitation is with the withdrawn blood and Ringer's lactate over 60 minutes. Their model exhibits a reduction in left ventricular function as measured by a reduction in the maximal rate of pressure in the left ventricle, ventricular peak systolic pressure and cardiac output. (15-21). Mechanistically, the group demonstrated that an estrus state attenuates the deleterious effects of trauma haemorrhage (TH) on cardiac function (15-25). They also showed that progesterone and castration mitigated the reduction in cardiac function through reduction in testosterone levels (15,26,27). Further

studies have implicated IL-6 in cardiac dysfunction in trauma, highlighting potential protection afforded by modulation of its downstream signaling leading to reduced production of various cytokines and cell adhesion molecules (28). These inflammatory cytokines were reduced by estradiols (21). Although, pre-clinical models have alluded to many associative factors in cardiac dysfunction in trauma, the absolute mechanism has not been clearly defined.

1.4 Clinical Evidence for Cardiac Dysfunction in Trauma

The clinical manifestation of cardiac dysfunction specifically evidence of myocardial ischaemia and arrhythmias have been previously studied in trauma patients but furthermore studies have investigated the relevance of cardiac biomarkers in trauma patients. These will be discussed later in Section 1.6.4.

In a retrospective case note study conducted by Ismailov et al., the incidence of acute myocardial infarction (AMI) in trauma patients was found to be 3%, and associated with thoracic injury, abdominal and pelvic injuries. Further examination through logistic regression revealed that in patients less than 45 years old, abdominal and pelvic injuries were associated with 65% increased risk of developing AMI. They also found that blunt cardiac injury was an independent risk factor for dysrhythmias, however, thoracic injury was not an independent risk factor. In this cohort, patients younger than 50 years had a four-fold increase in risk of developing arrhythmias (29). In younger patients with less likelihood of underlying ischaemic

heart disease and the increase in risk seen with abdominal and pelvic injuries potentially

Seguin et al conducted a prospective study into the occurrence of arrhythmias in critically injured patients. They reported the occurrence of Atrial Fibrillation (AF) at 5%, with 8% of those patients developing cardiogenic shock (30). Using multivariate analysis, this group also found that age, degree of shock, blunt thoracic injury and insertion of pulmonary artery catheters were all risk factors. Furthermore, they demonstrated that direct thoracic injury led to a 17-fold increase in risk. In 2006, the same group repeated the study to determine further risk factors, revealing a similar incidence of AF incidence (5.5%). However, in this study, thoracic injury was not an independent risk factor but shock, catecholamine use and number of regions injured were all risk factors when cofounders were adjusted for (31).

In a retrospective study conducted by Hadjizacharia et al in 2011, it was noted that the patients who developed atrial arrhythmias (AA) were older, more likely to be male, more severely shocked on admission and had a higher prevalence of blunt and head injuries. On multivariate analysis, being aged >55 years was the only independent risk factor. The AA group also had worse outcomes with a 19% greater risk of mortality. This study also noted that AA patients who received beta-blockers during their admission had 15% reduced risk of mortality (26).

1.5 Trauma Induced Secondary Cardiac Injury

In 2012, our group conducted a retrospective study on a panel of cardiac biomarkers in a cohort of 135 patients. In this study, 13% patients had adverse cardiac events (ACE). In the patients who developed ACEs, serum h-FABP levels were three times higher and BNP levels were 1.5 times higher and this was associated with greater injury severity and shock. The rise in cardiac biomarkers was not associated with thoracic injury nor head injuries. There was also no difference in pre-existing diseases such as hypertension, hypercholesterolaemia, diabetes and ischaemic heart disease. This raised the possibility of further underlying mechanisms for secondary cardiac injury in trauma patients(32).

In a follow up study, De'Ath went on to look at the association of inflammation in ACEs (33). In this study, patients with higher levels of circulating inflammatory cytokines – tumor necrosis factor (TNF) α, interleukin (IL)-6 and IL-8 had a hundred-fold increase in serum heart fatty acid binding protein (h-FABP). Multivariate analysis also revealed that age, injury severity score (ISS) and IL-6 were independent predictors of myocardial damage, as predicted by h-FABP. This study also found that patients admitted with raised levels of troponin I and elevated inflammatory cytokine had increased risk of mortality(33).

This was the first study designed to examine the possibility of identification of cardiac injury and prognostication in trauma patients using a panel of biomarkers.

Although retrospectively conducted, it demonstrated the rise of cardiac biomarkers in trauma patients and the association with inflammation. These studies successfully validated the preclinical evidence on cardiac injury in a clinical setting.

1.6 Cardiac Biomarkers

Myocardial injury is diagnosed using a combination of clinical features. It may manifest clinically as chest pain, palpitations, syncope or cardiovascular collapse. These are often seen in addition to electrocardiogram (ECG) changes such as ST elevation, arrhythmias, conduction abnormalities such as bundle branch blocks and T wave inversion. In the absence of information of patient's pre-existing co-morbidities or unequivocal clinical signs, cardiac biomarkers can aid diagnosis (34). The ideal biochemical marker should allow for early detection of cardiac injury with high sensitivity and specificity. A potential prognostic biomarker would therefore be a protein abundant in the myocardium with minimal or absence of expression in non-cardiac tissues, be released and detectable in blood stream, be stable enough for detection and be assayed in quick and cost-effective method.

1.6.1 Troponin- I

Troponin is a three part structural protein involved in the calcium mediated muscle contraction of the myocyte. It is made up of the calcium binding troponin C, the inhibitory (Cardiac Troponin I) cTnI (23 kDa) and the tropomyosin binding Troponin T (37kDa). Current assays are cardiospecific and targeted at the cardiac isoforms of

Troponin I and T. During development of myocardial ischaemia, the troponin subunits are released from the contractile apparatus and leak through the cell membrane, reaching peak concentrations in the serum around 6 hours (35). The advent of ultra-sensitive troponin measurement platforms has changed the clinical utility of troponin significantly. Due to their much lower limits of detection, AMI in patients with chest pain can be ruled out through serial measurements at 6 hours. A serum concentration of less than manufacturers reference value followed by less than 50 % increase on a serial measurement 6 hours later is considered to be an exclusion for AMI(36). Studies have also demonstrated its prognostic value in other conditions including pulmonary emboli (PE), sepsis and in the perioperative setting (37).

The development of right ventricular strain and cardiac dysfunction is a poor prognostic indicator in patients with pulmonary embolism. Raised cTnI levels in PE has been associated with cardiac dysfunction, increased rates of serious adverse event (SAE) and mortality (38,39). Identifying these patients for prompt thrombolysis has been suggested for improving patient outcomes (Beccattini 2007).

In sepsis, system inflammatory response syndrome (SIRS) and its associated hypotension, hypoperfusion and tachycardia have all been indicated to contribute to increased myocardial stress(8). In line with this, SIRS has also been shown to induce reversible as well as irreversible cardiac dysfunction (6). Furthermore, cTnI has been shown to be higher in patients admitted to intensive care unit (ICU) with

septic shock, and correlates with left ventricle (LV) dysfunction and associated poorer outcomes (40-43). A meta-analysis of 162 studies on the predictive value of cTnI on mortality in septic patients revealed that raised cTnI concentration was associated with a two-fold increase in mortality, however, it was not found to be an independent factor (44). It calculated the pooled area under the curve (AUC) on receiver operating characteristic (ROC) analysis as 0.68 indicating that cTnI is a good predictor of mortality in septic patients. In these studies, the manufacturers guided the serum concentration threshold for cardiac injury (44).

The myocardium is exposed to multiple deleterious factors during critical illness. Hypotension, hypoperfusion, fluid overload, circulating cytokines and tachycardia may all be experienced by trauma patients, and can increase the myocardial oxygen requirements of critically ill patients. Patients admitted to ICU have demonstrated higher levels of cTnI indicative of myocardial injury
(41,45,46). This underlying cardiac injury as indicated by the rise in serum cTnI levels does not always manifest clinically, yet it is associated with worse outcomes such as increased requirements for organ support, longer hospital stays and greater mortality (45).

1.6.2 Brain Type Natriuretic Peptide

Brain type natriuretic peptide (BNP) is a 37 kDa cardiac neurohormone that is systhesised in the ventricular myocardium and secreted into the bloodstream in response to myocardial stretch. Stretch stimuli leads to an immediate increase in its

expression and its release as a prohormone (pro-BNP), this is then cleaved into its active form BNP and inactive form NT-proBNP. The inactive form NT-proBNP has a longer half-life and therefore remains in the bloodstream longer than BNP. The active BNP acts on natriuresis, smooth muscle relaxation, inhibition of renin-angiotensin system and inhibition of sympathetic activity(47). BNP has been primarily used in the diagnosis of acute and chronic heart failure (48). It has been shown to be useful in the prognostication in AMI, with higher levels associated with greater ischaemic insult and left ventricular dysfunction (49,50). It has also been shown to predict postoperative MI, cardiovascular mortality and overall mortality in elective patient (51).

The increase in pulmonary venous pressure in PE leads to raised right ventricular pressure causing to myocardial stretch. Multiple prospective studies have shown that nt-proBNP correlates well with the development of serious adverse events in PE (39,52-57). A meta-analysis of studies investigating nt-proBNP in PE found the combined OR for mortality to be 6.2 (58). Higher levels were associated with increased rates of inotropic support, intubation and mortality. In a prospective study of 113 patients with PE, nt-proBNP was almost six times higher at 10,678 ng/ml in the non-survivors compared to 1895 in the survivors. A persistently raised nt-proBNP level, with circulating concentrations of >7500 ng/ml that remained raised at 24 hours was found to be the strongest predictor of mortality (56). The same research group went on to compare the efficacy of nt-proBNP and cTnI for risk stratification of normotensive patients. In their risk stratification model, they

found that patients with nt-proBNP of <600ng/ml did not develop any serious adverse events (SAE) and therefore could potentially be managed less aggressively (59).

In sepsis, an increase in serum BNP levels has been associated with echocardiographic changes of reversible and sustained cardiac dysfunction (60) (11) (61) (11,60,61). Raised BNP levels leads to an 8-fold increase in mortality in septic patients(62). It was noted to be a moderate predictor of mortality in sepsis with a AUC of 0.65 on ROC analysis. These raised BNP levels used in combination with clinical acumen could help delineate patients at greater risk of adverse outcomes. These results have also been reproduced in the general critical care patient population (63,64).

1.6.3 Heart Related Fatty Acid Binding Protein (h-FABP)

Heart related fatty acid binding protein (h-FABP) is a relatively new cardiac biomarker; first discovered as a marker of myocardial injury in 1988. It is a small 14kDa cytosolic protein that acts a transporter of fatty acids from the cell membrane to the mitochondria for oxidation. It is released as early as 90 minutes post myocardial ischaemia, reaching peak concentrations within the plasma at 6 hours. Values of 6-7 ng/ml are considered to be indicative of cardiac ischaemia. In AMI, it has been shown to exhibit greater sensitivity in comparison to troponin with a better negative predictive value, however due to its lack of specificity, troponin remains the gold standard for the diagnosis of AMI. Furthermore, Cardiac TnI was

superior at predicting AMI in patients presenting with chest pain in comparison to h-FABP (AUC on ROC analysis 0.82 vs. 0.78). However, due to its greater sensitivity, h-FABP had a better negative predictive value but this made it lower specificity (65). The lower specificity may be owed to its expression in other tissues including skeletal, brain, lung and kidney, albeit in much lower concentrations(66). The role of h-FABP has been investigated in prognostication of acute coronary syndrome (ACS) and has been shown to be effective in predicting adverse cardiac events and death (67-69).

Similarly to cTnI and BNP, h-FABP has also been shown to be a potentially valuable tool in prognostication. In PE, it has been shown to be raised in patients who develop adverse outcomes and those who die within 30 days of admission (70-73). Raised h-FABP levels on admission was related with a 17-fold increased risk of developing adverse outcomes and 33-fold risk of mortality (74). Higher levels of h-FABP were also associated with right ventricular dysfunction as seen on echo cardiogram(70,71). In a meta-analysis comparing the three biomarkers, h-FABP was shown to have a greater propensity for predicting adverse outcomes and mortality in PE in comparison to troponin and BNP (75,76). A retrospective analysis of h-FABP levels in intermediate risk PE patients, thrombolysed according to current guidelines, did not show better outcomes between the groups who were h-FABP positive and those who were not (77). In combination with clinical acumen, its value mostly lies in ruling out those at low risk, rather than guiding treatment.

In sepsis, h-FABP levels on admission to critical care units (CCU) predict development of organ dysfunction and mortality with greater accuracy than cTnI. Patients with higher h-FABP levels required earlier mechanical ventilation and inotropic support (78). There was no difference in h-FABP levels In those who required organ support overall (78,79). In an emergency department setting, a study of 99 patients with severe sepsis showed h-FABP levels to be predictive of ICU admission (80). Due to the differential distribution of h-FABP in other tissues, it has been postulated as a marker of global ischaemia in critical illness, however due its low expression in other tissues, it may only be an indirect marker (78).

1.6.4 Cardiac biomarkers in trauma

1.6.4.1 Troponin in Trauma

Cardiac biomarkers have been used to evaluate direct and indirect cardiac injury in trauma patients. In 2012, Eastern Association for the Surgery of Trauma published guidelines on blunt cardiac injury which included diagnosis and management based on clinical suspicion, ECG changes and cTnI levels. On review of the current evidence, they recommend that blunt cardiac injury (BCI) can be ruled out if ECG and troponin I are normal. In studies that looked at BCI, cTnI levels of 1.05- 2ng/ml were found to have good predictive value of cardiac injury and were therefore suggested as a prognostic marker to guide the monitoring requirements of trauma patients (81-83).

In a large study of 728 patients, Edouard et al., demonstrated that cTnI was raised in 12% of their trauma population unrelated to blunt cardiac contusion. In the cTnI positive group, 8% were due to underlying coronary artery disease. In the cTnI patients without pre-existing co-morbidities, there was a 29% mortality rate within the first 48 hours compared to 3% in the cTnI negative group. On subgroup analysis, release of cTnI, was found to be associated with chest and head injury.

In a study of 89 patients with mild trauma, it was shown that patients with raised troponin I levels were more likely to develop cardiac complications unrelated to pre-existing cardiac comorbidities. However, the nature of the patients' injuries and particularly the presence of chest injuries was not explored in this study (84).

In a large retrospective study of trauma patients to identify risk factors for the development of cardiac injury in trauma patients who develop sepsis, patients who had troponin measurements during their admission were identified. The study of this population revealed that a pre-existing diagnosis of chronic obstruction pulmonary disease (COPD), injury severity, ventilator acquire pneumonia (VAP) and urinary tract infection (UTI) were all risk factors for the development of indirect cardiac injury in these patients. When the hazard ratio was adjusted for age, UTI posed a greater risk in those above the age of 8. Due to the retrospective nature of the study, not all patients had troponin ordered, only those that at the time were deemed to have signs of cardiac ischaemia (85)

In a paediatric study looking at direct cardiac injury, 27% of the patients had raised cTnI but none of these patients had abnormal ECGs and 25% had changes on ECG, however, they did go on to have higher rates of mortality. Their raised troponin levels were related to a greater degree of hypotension and the group hypothesised that this may have been an indicator of global hypoperfusion leading to myocardial ischaemia rather than direct injury (86).

1.6.4.2 BNP in Trauma

In a small prospective study of 26 patients, nt-ProBNP was shown to be raised in critically injured patients and correlated with MODs and a reduction in cardiac index. Although, this study did not comment on role of direct mechanical injury in the release of BNP, all of the patients had significant thoracic injuries (87).

Firese et al, conducted a prospective study on 134 trauma patients which showed that BNP levels rose with fluid resuscitation and was higher in those who went on to develop pulmonary oedema. However, the serum levels did not cross the threshold of 100 pg/ml seen with ACS (88). Surprisingly, the volume of fluid infused did not correlate with BNP levels, which was further corroborated by a smaller study where the degree of haemorrhage nor the volume of fluid used in resuscitation correlated with BNP levels. (88,89).

A further study by Friese's group in 2010 also demonstrated that an increase in BNP >75ng/ml over 24 hours was predictive of the need for mechanical ventilation (90). On investigating the prognostic value of BNP in trauma, Stewart et al. demonstrated

that BNP did not correlate with LV ejection fraction (LVEF), length of ICU stay or hospital stay. Patients who had evidence of LV dysfunction on echocardiogram did not have higher levels of BNP (91).

1.6.4.3 H-FABP in Trauma

Due to the relative novelty of H-FABP, its use in trauma studies has been limited. In a prospective study of 55 poly trauma patients with direct thoracic injury, h-FABP levels were higher in patients with thoracic injuries but not in patients with cardiac injury. However, the author does not clarify how cardiac injury was defined, especially only 41% of patients had abnormalities on ECG or echocardiograms (92). The use of h-FABP as a cardiac biomarker will be confounded by its expression in brain tissue. In a small but insightful study conducted by walder et al, they shed some light on the contribution of traumatic brain injury (TBI) to the release of h-FABP. In this prospective study of 45 patients, although, h-FABP was elevated in both the isolated TBI as well as TBI associated with polytrauma, the levels were 1.5 higher in the polytrauma group at 6 hours, 3 times higher at 24 hours and 4 times higher at 48 hours. Furthermore, the presence of concurrent cardiac complication in the isolated head injury group was not explored in this study (93). This differential rise in H-FABP in addition to clinical signs, ECGs and echocardiogram, lend to its potential usefulness as an early cardiac biomarker in trauma.

1.7 Catecholamines and Adverse Cardiac Events

The stresses of events preceding traumatic injury in combination with the severe injuries sustained by trauma patients evoke a significant sympathetic response and therefore a catecholamine surge. In this study, the cardiac effects of the catecholamines release will be explored. Similar studies in septic patients have shown raised catecholamine levels. The adrenergic response in severe sepsis has been shown to induce myocardial cell necrosis and changes to intracellular calcium concentration. Intracellular calcium depletion secondary to increased cellular stress causes disruption to the mitochondrial activity and leads to apoptosis and necrosis. (8,94). In patients with subarachnoid haemorrhage, catecholamine surge associated with significant injury has been noted to lead to cardiac injury and dysfunction (95) with evidence for pre-hospital beta-blockade showing mixed outcomes (96-99).

1.8 Pathological changes in the Myocardium following trauma haemorrhage

Forensic pathologists have expressed academic interest in histological changes of the myocardium following trauma for decades. In 1980, a case series of the postmortem histological examination of 15 homicidal victims was published. This showed myofibrillar degeneration in 11 of the 15 patients which the authors attributed to stress induced catecholamine release (100). In a further postmortem study of 24 trauma patients, myocardial histology revealed the presence of

contraction bands, myofibrillar fragmentation and positive staining for calmodulin, used as a marker of early ischaemia (101).

More recently, a large human study looking at histological changes of the myocardium in trauma patient hearts postmortem has demonstrated both macroscopic as well as microscopic architectural changes. Examination of the myocardium of 125 trauma patients demonstrated a variety of histological changes in keeping with ischaemia and necrosis including congestion, interstitial edema, fragmentation of myocytes, haemorrhage, leukocytic infiltration, coagulative necrosis, hyaline degeneration of myocytes, cytoplasmic vacuolation, collagenisation of myofibers, hypertrophy of few myocytes and focal mild interstitial fibrosis. However, It is difficult to assign causality, as the effect of mode of death and pattern of injury was not analysed alongside the histological changes (102). Regardless, this is an impressive study and the first of its kind to provide such large amounts of histological data on the effect of trauma on the human hearts.

Animal studies have demonstrated that traumatic injury leads to cardiomyocyte apoptosis(103,104) (103); (104). Mechanistic studies have elucidated a TNF-α driven, caspase dependent pathway as the cause for this myocardial apoptosis (103). In rat models, 2 hours of TH has been shown to be associated with increased expression of the pro-apoptotic protein Bax and reduction of the anti-apoptotic protein Bcl-2, leading to increased cardiomyocyte apoptosis as seen on H&E. Furthermore, this correlate with creatine kinase (CK) and lactate hydrogenase (LDH) as biomarkers of cardiac injury. This study also demonstrated that administration of

hydrogen sulfide, a regulator of mitochondrial transition pore opening, reduced the deleterious effects of TH serum (104). The involvement of the mitochondria in the reduction of cardiac function was corroborated by a murine study demonstrating concurrent reduction in mitochondrial function with reduced adenosine triphosphate (ATP) production, reduced expression of mitochondrial respiratory complex (MRC) proteins, increased release of cytochrome C into the cytosol and increased caspase-3 cleavage (105).

Ischaemic injury of the heart has been associated with myocardial necrosis (106). More recently, the processes that define different cell death mechanisms have been noted to cross over (107). Although, there has been interesting insight into cardiac dysfunction and cell death, we are still unclear as to what pathways are involved.

1.9 Cell Death Pathways

The release of cardiac structural proteins such as cTnI and h-FABP requires disruption of the cellular membrane. Tissue injury releases damaged deoxyribose nucleic acid (DNA), toxins and inflammatory proteins. These can all act directly on the cell, potentially disrupting its membrane, leading to necrosis or initiation of cell death via the extrinsic or intrinsic pathways of apoptosis.

A cell is considered dead if it has undergone irreversible permeabilisation of its cell membrane and complete fragmentation. Although, historically, cell death was

thought to occur either by necrosis or apoptosis, it has become apparent that the fate of a dying cell is not so binary in nature. It can however, be divided into accidental cell death and programmed cell death. Accidental cell death occurs following significant direct exposure to severe adverse conditions such as toxins, chemicals, temperature or mechanical injury. The noxious insult itself leads to cell membrane lysis, hence oedema of cell organelles. The pathway of the programmed cell death processes and the final fate of the cell is dependent on the trigger, the initial site of activation and concurrent activation of other pathways. The pathways are not always linear with many points of cross talk {Galluzzi:kj}.

1.9.1 Apoptosis

Apoptosis described as a programmed cell death mechanism is thought to reduce collateral damage by the formation of apoptosomes, which are then cleared by phagocytosis. On electron microscopy, apoptosis is characterised by deep staining of the nuclear mass (pyknosis) and formation of condensed cell bodies (apoptotic bodies). Apoptosis can be further differentiated to death receptor driven (extrinisic/type I) or mitochondria driven (intrinsic/type II).

1.9.2 Extrinsic Death Receptor Pathway

The extrinsic pathways are dependent on activation of death domain containing receptors. The two well-studied receptors are Fas Ligand receptor and TNF Receptor (TNFR). The activation of these receptors leads to cleavage of procaspase 8 to caspase 8, and in turn the activation of caspase 3 and 7 and thereon the proteolytic cleavage of proteins which leads to apoptosis (106). TNFα upregulation

is well reported in trauma so extrinsic pathways of apoptosis may well be at play. In vitro studies have shows that TNFα antagonism reduces cell death in cardiomyocytes incubated in trauma serum (103).

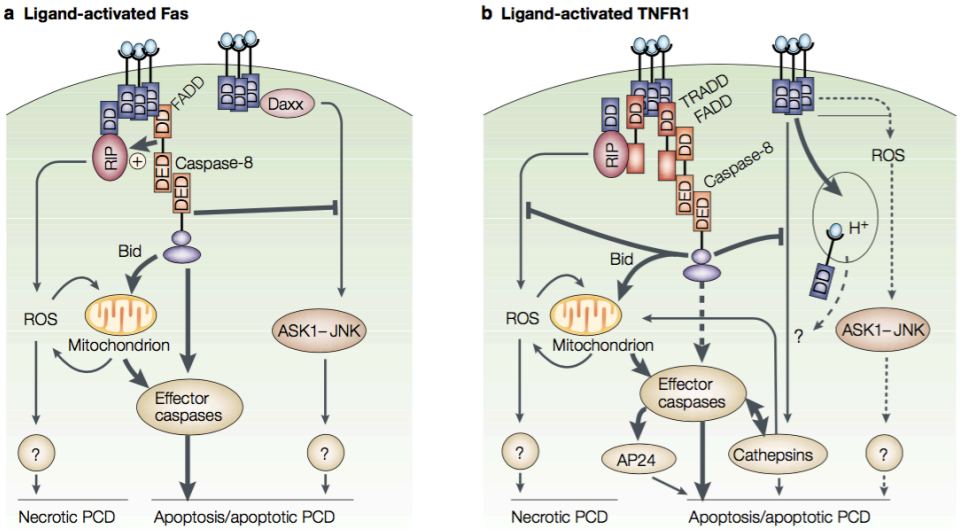


Figure 1.2 *Schematic diagram of the extrinsic pathway of apoptosis highlighting Fas and TNF receptor signaling and associated downstream players (107)*

1.9.3 Intrinsic Cell Death Pathways

The intrinsic cell death pathways are initiated by a combination of external and internal stimuli. A delicate balance of pro-apoptotic and anti-apoptotic the Bcl-2 proteins govern the intrinsic pathways. Under physiological conditions, the anti-apoptotic proteins of the family maintain the integrity of the mitochondria. Noxious external stimuli and metabolic disturbance within the cell, for example release of reactive oxygen species (ROS) or excess calcium, leads to activation of BH3 proteins and its subsequent its translocation from the mitochondria to the cytosol. In the cytosol, BH3 proteins act to either directly antagonise pro-apoptotic proteins or

activate the apoptotic proteins Bax and Bak. Activated Bax and Bak oligomerise, leading to their conformational change and insertion in to the outer mitochondrial membrane where they form pores. The changes in mitochondrial permeability and concurrent membrane potential can lead to three outcomes; influx of calcium and ROS into the cell directly leading to necrosis, release of cytochrome C causing induction of apoptosis through caspase 9 dependent pathways, or the release of caspase independent death- associated protein AIF-1 (Apoptosis inducing factor 1)(106).

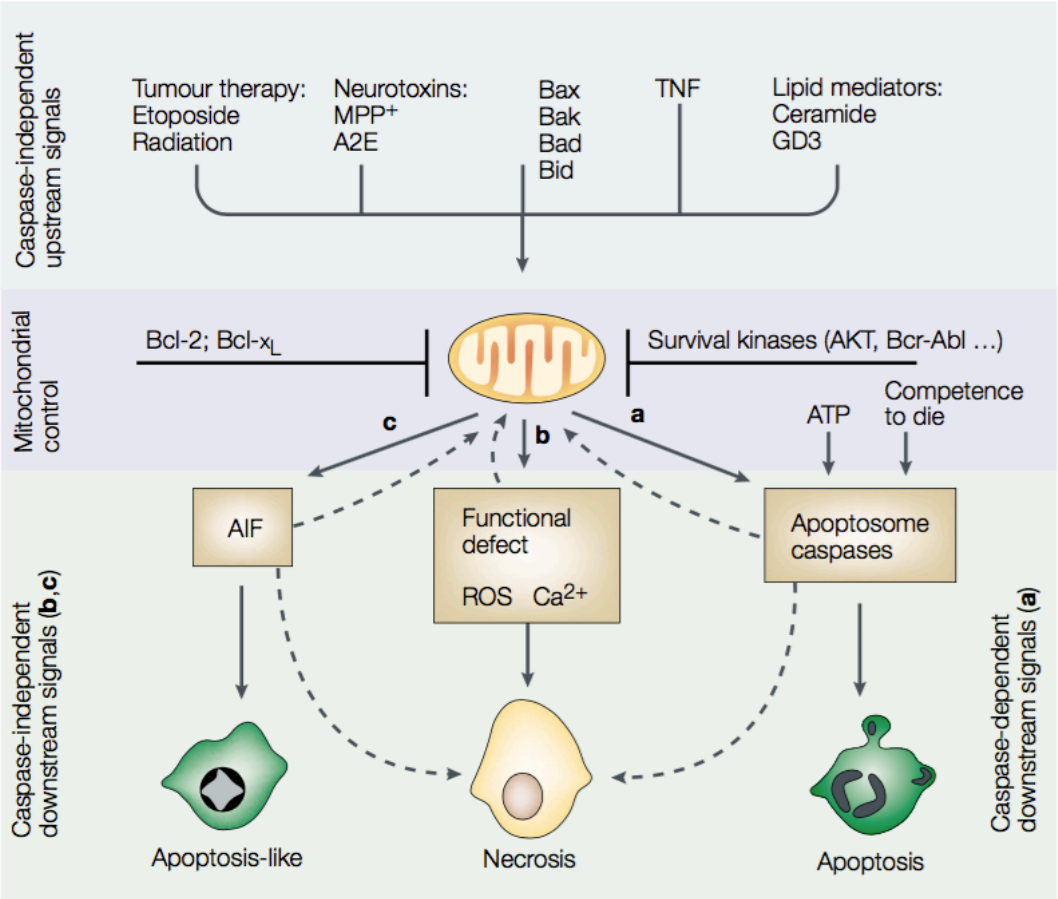


Figure 1.3 *Schematic diagram of the intrinsic pathways of apoptosis demonstrating the central role of the mitochondria in inducing cell death(107)*

In trauma as described above, the sequelae of events following the initial impact of the injury occur due to bleeding and tissue damage. Significant bleeding leads to poor perfusion, hypoxia, anaerobic respiration and acidosis, which can all lead to the activation of the intrinsic pathway. The role of Bcl-2 has been highlighted in improving immune cell survival in trauma haemorrhage and in apoptosis in haemorrhagic shock (108). In a small clinical study, Bcl-2 was seen to rise by 50% on the second day in comparison to admission and was related to increased infection rates (109)

1.9.4 Apoptosis Inducing Factor

Apoptosis inducing factor (AIF) is a mitochondrial flavoprotein that exists in the intermembrane space attached to the mitochondrial inner membrane. The nuclear coded AIF gene gives rise to a 67kDa protein with a mitochondrial localization sequence. Maturation of the precursor leads to the cleavage of mitochondrial localization sequence (MLS) and its attachment to the mitochondrial inner membrane. Apoptotic signaling leads to its further proteolytic cleavage causing its release from the mitochondrial inner membrane in its 57 kDa truncated form. The activation of the intrinsic cell death pathway by Bcl-2 related proteins leads to the permeabilisation of the mitochondrial outer membrane releasing the apoptogenic AIF into the cytosol, which then translocates to the nucleus where it leads to peripheral chromatin condensation, DNA fragmentation and blebbing associated with irregular shaped nuclei. Susin et al demonstrated these characteristic apoptotic nuclear changes in healthy heLA cell nuclei incubated in medium containing AIF (110). Although apoptosis occurred in the absence of cytosolic

proteins, once the nuclei was heat treated, the apoptotic changes were not seen, suggesting the need for nuclear proteases to aid AIF in its apoptogenic actions.

1.10 Rationale for Research

The retrospective studies conducted by De'Ath in addition to the previous studies showing the prognostic value of cTnI, BNP and h-FABP in traumatic injury provide good founding evidence for the presence of myocardial injury in traumatic injury. Through prospective design and analysis, the objective of work in this thesis is to further investigate the pattern of cardiac injury and try to delineate its causes and underlying mechanisms driving these effects.

Cardiac biomarker detection in trauma patients is complicated by the presence of multiple organ injury, tissue injury and hypoperfusion. In order to explore the impact of trauma directly on cardiac tissues, a murine model of trauma will be used to allow direct examination of cardiac tissue following trauma haemorrhage through the use of microscopy and protein analysis.

1.11 Study Aims

The overarching goal of this thesis is to further the understanding of trauma induced secondary cardiac injury (TISCI). For this purpose, this study aims to:

1. Describe the clinical manifestations of TISCI, potential risk factors and its effect on outcomes
2. Identify potentialbiomarkers of TISCI
3. Determine whether there is a potential role for catecholamines in the pathogenesis of TISCI
4. Determine the cellular mechanisms driving myocardial injury in TISCI

Chapter 2

Methods

2.1 Introduction

This chapter summarises the materials and methods used in Chapters 3-5. The clinical study methodologies apply to bother chapters 3 and 4 as well as the principles of enzyme-linked immunoassays (ELISA). It is presented separately rather than within each chapters for ease of reference and to avoid repetition. Chemicals and reagents used throughout the thesis are listed in this chapter, alongside the manufacturer and catalogue number. All were of biological or analytical quality.

2.2 Prospective Observational Study- TACID

Following a retrospective review of patients, which demonstrated a rise in cardiac biomarkers, and an increased incidence of cardiac events, Trauma Associated Cardiac Injury and Death (TACID) was designed to prospectively validate this.

2.2.1 Study Design

TACID was designed as a single centre, prospective, observational cohort study performed at Royal London Hospital, a major, level I, urban trauma centre in the United Kingdom.

The study was reviewed and approved by the Cambridge 3 Research Ethics Committee. (Reference No. 10/H0306/47)

2.2.2 Study Population

All adult patients above aged 16 and above, who triggered the trauma team activation and presented with an abnormal primary survey were screened for recruitment in the study. Patients were assessed by research personnel and enrolled between the hours of 8:00 and 20:00 if found to have an abnormal primary survey.

Exclusion criteria were:

- Patients < 16 years of age
- Patients transferred from other hospitals
- Patients not expected to survive > 72 hours
- Pregnant
- Prisoners
- Penetrating cardiac injuries
- Trauma team leader deemed recruitment inappropriate
- Patients were retrospectively excluded if they or a personal consultee declined to give consent for the research study

2.2.3. Consent

Due to the severity of their injuries most of our patients are incapacitated on admission. A system approved by the research ethics committee was established in order to gain consent for the recruitment of these patients in this study.

On admission, the emergency department (ED) consultants who were familiar, but independent to, the study act as the patients' advocate and the Professional Legally

Appointed Representative (PLAR). Once consent is granted by the PLAR the patient can be ethically recruited to the study, and subject personal (next of kin) or patient consent can then be sought retrospectively in a time dependent manner. Patients are assessed daily to determine if they have capacity. If the patient is not deemed to have capacity, consent is sought from their next of kin who act as the patient's legally authorised representative (LAR).

Patients and their next of kin are provided with detailed study information sheets and approached after allowing time for them to consider their involvement in the study. The patient is withdrawn from the study if either the patient or next of kin refused consent. If the patient is permanently incapacitated they are able to remain in the study under LAR consent. Patient who die acutely or prior to receipt of appropriate LAR consent from a next of kin can remain enrolled in the study under PLAR consent. A database logging all consent attempts was maintained to document adherence to these consent procedures.

2.2.4 Data Collection

Data was collected prospectively by the research personnel on patient demographics, mechanism of injury, injury time, time of arrival in the ED, baseline vital signs, daily physiological data, Injury Severity Score (ISS), Abbreviated Injury Scale (AIS), past medical history and concomitant cardiac medication. The patients were reviewed daily to record any occurrence of cardiac events during admission, the length of stay and mortality. Patients with adverse cardiac events had echocardiograms.

2.2.5 Blood sampling from trauma patients

Within 20 minutes of arrival to ED, patients had 10 millilitres of blood drawn from the femoral vein or antecubital vein in addition to clinical bloods. Blood was drawn into a 4·5-ml Vacutainer® containing 3.2% (0·109 mol/l) buffered sodium citrate (Becton Dickinson, Plymouth, UK). Blood was collected on admission, 24 hours following admission and 72 hours following admission. These time points were chosen to help identify immediate and late biomarkers of cardiac injury.

2.2.6 Sample Handling

The citrated blood was centrifuged for 10 minutes at 1750g within 1 h of collection. Plasma was collected and then centrifuged further for 10 minutes at 1750g before being transferred into 0·6-ml Eppendorf tubes and subsequently frozen at −80˚C until ready for processing. Plasma stored at -80^{o}C was thawed to 37^{o}C prior to biochemical analysis.

2.3 Enzyme Linked Immunosorbent Assays

2.3.1 Technique

Enzyme-linked immunosorbent Assay (ELISA) is a quantitative immunoassay for the measurement of an analyte of interest in a liquid sample. In biological samples, it is used to detect antibodies, antigens, proteins and glycoproteins(111).

The ELISAs are carried out on 96-well plates often pre-coated with ligand binding specific reagent and dried to form the solid-phase. The cardiac biomarkers were all measured using sandwich ELISAs and the Catecholamines were measured using competitive ELISAs.

In a sandwich ELISA, the plates are coated with a capture antibody, specific to the protein of interest. The sample along with controls of known concentration of the antigen of interest is incubated in the wells of the plate. Any antigen specific to the antibody will bind and after incubation, any excess unbound antigen is washed and subsequently a detection antibody is added. In the following ELISAs, the detection antibody was attached to horseradish peroxidase. Following incubation, the excess is washed and a chromogenic substrate such as tetramethylbenzine (TMB) was added. After 15-20 minutes of incubation; an acid is added to cease the reaction. This is an indirect visualisation method as the chromogen does not directly bind to

the antigen of interest but to its corresponding detection antibody, thereby increasing the specificity of the assay (111).

In a competitive ELISA, the sample is initially incubated with a specific antibody for the antigen of interest. Then this sample, now containing antigen- antibody complexes is added to the wells coated with purified antigen of interest. Any unbound antibody will bind to the antigens on the wall of well. So higher the concentration, the lower the remaining amount of free antibody. Then detection antibody is added, followed with a chromogen and stop substrate as above(111).

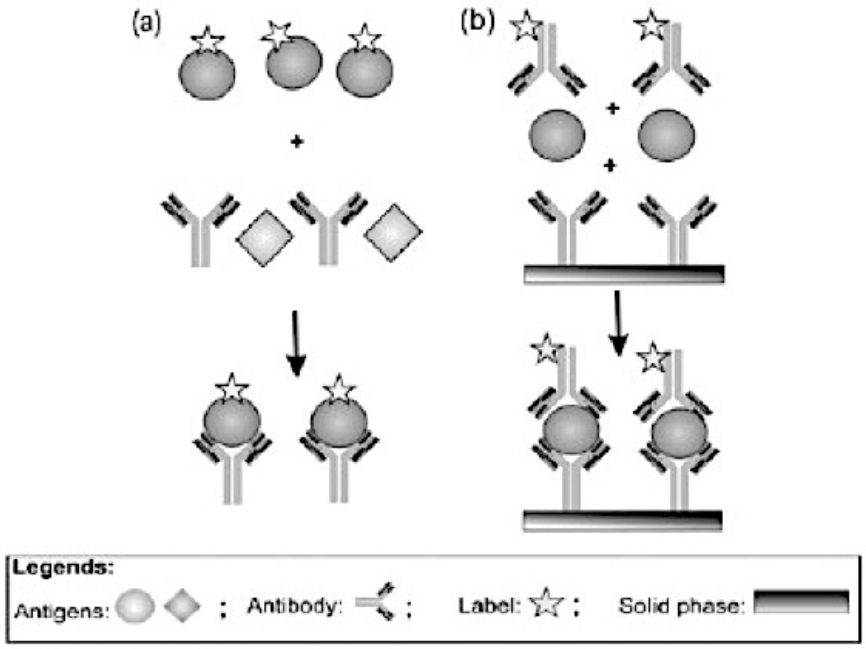


Figure 2.1*: Schematic diagram of enzyme-linked immunosorbent assay formats- (a) Competitive ELISA and (b) Sandwich ELISA (112)*

Sandwich ELISAs are extremely sensitive due to the utilisation of two antibodies and they allow for accurate determination of concentration of an analyte in a crude sample, even without prior purification. The competitive ELISAs are useful in

detecting small substrates, particularly when they only have a single antibody binding site(111).

2.3.2 Materials

All ELISAs were commercially manufactured, ready-to-use kits with standard, plates and reagents all included. Limits of detention and normal reference range as specified by the manufacturers have been summarised.

Table 2.1 Manufacturers of Biomarker ELISAs, limits of detection and normal reference values

Biomarker	Manufacturer	Limits of Detection	Manufacturers' Reference
H-FABP	*Hycult Biotech* Cat No. HK402	0.1-25 ng/ml	<1.6 ng/ml
Nt-ProBNP$_{8-29}$	*Biomedica,* Cat No. BI-20852W	0-6400 pmol/l	<392 pmol/l
Troponin I	*Signosis* Cat No.	0.1- 25 ng/ml	<0.1 ng/ml
Catecholamine	*Diasource* Cat No. KAPL 10-1600		
Noradrenaline		0- 0.05 ng/ml	<0.1 ng/ml
Adrenaline		0- 0.01 ng/ml	<0.6 ng/ml
Dopamine		0- 0.025 ng/ml	<0.1 ng/ml

2.3.3 H-FABP ELISA

1. 96 well plates used were labelled appropriately on a matching schematic diagram along with appropriate Eppendorf tubes.
2. 50 µl of diluted peroxidase-conjugated second antibody was added to each well.
3. 50 µl in duplicate of standard, samples, or controls into appropriate wells.
4. The plate was covered and incubated for 60 minutes at room temperature
5. The plates were washed 4 times with wash buffer using a plate washer with 200ul of wash buffer.

6. 100 µl of TMB substrate was added to each well.
7. The tray was covered with foil and incubated for 15 minutes at room temperature.
8. The reaction was stopped by adding 100 µl of stop solution
9. The solutions in the wells were mixed thoroughly by gently swirling the plate.
10. The plate was read within 30 minutes after addition of stop solution at 450 nm using a plate reader

2.3.4 BNP ELISA

1. 96 well plates used were labelled appropriately on a matching schematic diagram along with appropriate Eppendorf tubes.
2. Standards of predetermined concentrations were reconstituted with distilled water.
3. 150 µl of assay buffer is added to all wells expected the blank. This is followed by 30 µl of the standard or sample in its respective wells.
4. This is followed by 50 µl of conjugate into each well except the blank.
5. The plate is covered in foil and incubated overnight (16-25 hours) in the fridge at $4^{o}C$
6. The plate was washed using a plate washer. 300 µl of washer buffer was used per wash and this was repeated five times. On the last cycle all wells are aspirated
7. Following this, 200 µl of substrate is added to wash well and the plate incubated at room temperature for 20 minutes.
8. 500 µl of stop solution is then added to each well.
9. Absorbance is measured at 450nm. The blank OD is subtracted from the values of the standard, control and sample.
10. A standard curve was constructed using the OD values of the standards.
11. The control was used to verify the standard curve. The acceptable range for the concentration of the control is provided per kit.

2.3.5 Troponin I ELISA

1. 96 well plates used were labelled appropriately on a matching schematic diagram along with appropriate Eppendorf tubes.
2. The standards provided as 0, 2, 7.5, 30 and 75 ng/ml were reconstituted using 1 ml of distilled water and left to stand for 20 minutes.
3. 100 µl of standard or sample was added to each well followed by 100 µl of enzyme conjugate reagent.
4. After mixing on a plate shaker for 30 seconds, the plate was left to incubate for 90 minutes.
5. The mixture is aspirated and the plate washed in a plate washer with 300 µl of diluted wash buffer and the process repeated 5 times. The wash buffer is aspirated following the last wash.
6. 100 µl of TMB is added to each well and placed on the plater shaker for 10 seconds and left to incubate for 20 minutes.
7. Then reaction is halted by the addition of 100 µl of stop solution to each well and the plate is placed on the shaker for 30 seconds till all wells have gone from blue to yellow in colour

2.3.6 Catecholamine ELISA

2.3.6.1 Acylation of samples

1. 96 well plates were labelled appropriately with corresponding schematic diagrams and eppendorf tubes.
2. 10µl of standards and 300µl of samples were added to each well.
3. 250µl Distilled water, 50µl Assay buffer and 50µl Extraction buffer
4. The plate was incubated for 30min at RT on shaker at 500rpm
5. Supernatant was removed and 1ml wash buffer into each well and the plate was incubated for 5min at room temperature on shaker at 500rpm
6. Supernatant was removed and 1ml wash buffer into each well and incubated for a further 5min at room temperature on the shaker at 500rpm
7. Supernatant was removed and the plate blotted dry

8. 150µl of acylation Buffer was added followed by 25µl of acylation reagent and incubated for 15min at room temperature on a shaker at 500rpm
9. The supernatant was removed and the plate blotted dry
10. 1 ml wash buffer was added into each well and the plate incubated for 10min at room temperature on shaker at 500rpm
11. The supernatant was decanted and the plate was blot dried.
12. 175µl of HCL was added and incubated for 10min RT on shaker at 500rpm

2.3.6.2 Competitive Catecholamine ELISA

1. 25µl of reconstituted enzyme solution was added into each well
2. 25µl standards and 50µl Samples were added (along with 25µl HCL into each well for the dopamine ELISA)
3. The plate was incubated for 30minutes at room temperature on shaker at 500rpm
4. 50µl of the appropriate antiserum (Dopamine, noradrenaline or adrenaline) was added.
5. The plate was incubated for a further 120 minutes at room temperature on a shaker at 500rpm
6. Plates were washed using a washer, 3 times with 300µl of wash buffer in each well
7. 100µl Enzyme conjugate into each well and incubated for 30 minutes at room temperature on a plate shaker at 500rpm
8. The plate was washed again 3 times with 300µl of wash buffer in each well
9. 100µl of substrate was added and the plate covered with foil and incubated for 30 minutes at room temperature on a shaker at 500rpm
10. The plate was washed 3 times with 300µl of wash buffer in each well
11. 100µl stop solution was added and read at 450nm

2.3.7 Quantification of results

The samples were read at 450nm on a plate reader. A standard curve was produced from the controls with known values using GraphPad Prism. This generates a curve fit by plotting the mean absorbance for each standard concentration on the vertical (Y) axis and the corresponding concentration on the horizontal (X) axis on a logarithmic axis. The concentrations read from diluted samples were multiplied by the dilution factor. Any sample with concentration variance between duplicates greater than 15% was repeated.

2.4 Animal Models

The animal model for TISCI was developed by *Dr Johanna Wall* as part of her doctoral thesis. The methodology has been reproduced with her permission.

All animal procedures described in this thesis are regulated under a specific animal project licence (Procedure Project Licence (PPL) – PC5F29685) approved by the Animal welfare and Ethical Review Body at Queen Mary University of London, and the UK Home Office, in accordance with the EU directive 2010/63/EU on the protection of animals used for scientific purposes. The PPL includes all the experimental procedures required for the pre-clinical studies, including the induction of haemorrhage and traumatic injury, imaging procedures, dosing substances and blood / tissue samplings. This PPL was renewed on December 2016 and has been granted for 5 years (to December 2021).

2.4.1. Mice

Male C57BL/6 wild-type mice weighing 25 – 30 grams were supplied by Charles River Laboratories (Margate, UK) and housed in accordance with the UK Home Office Guidance in the Operation of Animals (Scientific Procedures) Act 1986 and received a standard diet and water ad libitum prior to undergoing anaesthesia. Mice were randomly allocated to experimental group.

2.4.2 Anaesthesia

Isoflurane (Abbott Labs ltd, Berkshire, UK) in combination with 100% medical oxygen was continuously delivered via nose cone for both induction and maintenance of general anaesthesia throughout the duration of the experiments. All animals were culled at the end of the experiment whilst still anaesthetised.

2.4.3 Surgery

After induction of anaesthesia, the left jugular vein and carotid were cannulated. A pressure transducer was connected to the line by way of a three-way connector. Pressure-controlled haemorrhage of arterial blood via the carotid catheter was conducted over 10 minutes to achieve a target MABP of 60 – 70mmHg or 30 – 40mmHg (depending upon the experimental group allocation). The target blood pressure was then maintained over a 60-minute period. To produce truamtic injury in the mice models bilateral hind limb fracture, laparotomy and rectus muscle crushing were carried out. Fractures were performed using a closed, manual 3-point bending technique. A 2cm midline laparotomy was performed, with internal inspection of the abdominal viscera in order to exclude inadvertent iatrogenic injury

and / or bleeding, followed by crushing of the rectus muscle and then the laparotomy was closed using 5.0 monofilament suture material.

2.4.4 Observation periods

After trauma-haemorrhage and resuscitation episodes, animals underwent a 60-minute observation period. During this time, core temperature was continuously monitored and maintained with the use of a heat mat and lamps. Temperature, MABP and % of inhaled anaesthetic required to maintain a surgical plane of anaesthesia were recorded at 10-minute intervals.

Table 2.2 Summary of conditions of models

Sample	Time Of Death	Conditions	Mortality	EM	WB	HFABP
R43	3h	Sham	N			17.2
R44	3h	Sham	N	X	X	14.64
R68	3h	Sham			X	3.3
R69	3h	Sham			X	3.4
R76	3h	Sham	N	X	X	3.91
R77	3h	Sham	N	X	X	38.26
R90	3h	Sham	N		X	3.33
R91	3h	Sham	N		X	3.48
R41	3h	TH	Y	X		334.28
R42	3h	TH	Y	X		(unable to bleed)
R62	3h	TH	N	X	X	(unable to bleed)
R64	3h	TH	N	X	X	432.60
R78	3h	TH	N		X	242.36
R79	3h	TH	N		X	193.57
R81	3h	TH	Y		X	76.43
R65	6h	TH	Y	X	X	386.26
R66	6h	TH	N	X	X	417.34
R67	6h	TH	N	X	X	433.22

2.5 Histology

2.5.1 Technique

Immunohistochemistry is a useful tool in visualisation expression of a protein and its pattern of localisation in tissue. Specific antibodies targeting epitopes of interest are attached to chromogens to allow identification. The advent of fluorescence immunohistochemistry has made it possible to label multiple proteins simultaneously.

Tissues must be preserved and fixed to maintain their structure and avoid degradation of proteins. Samples can be formalin fixed, fresh frozen or paraffin embedded. Initially, I attempted fresh frozen sectioning of the cardiac tissue as it retains antigenicity better, however, the morphology was compromised, therefore, experiments were completed using paraffin embedded sections.

2.5.2. Antibodies

Mouse Monoclonal Anti-cardiac Troponin I (Abcam ab10231)

Rabbit polyclonal Anti-AIF antibody (Abcam ab2086)

Rabbit Monoclonal Anti-Cleaved Caspase 8 (Cell Signalling 8592S)

AntiRabbit IgG (Alexa Fluor® 488) (Abcam ab150129)

Donkey Anti-Mouse IgG (Alexa Fluor® 594) (Abcam ab175774)

2.5.3 Tissue Handling

Hearts were excised whilst animals were anaesthetised and immersed into 4% paraformaldehyde and stored till enough samples were available for mass processing.

2.5.4 Sectioning

Tissues were dehydrated with 70% ethanol three times for 30 minutes each at room temperature, 90% ethanol two times for 30 minutes each at room temperature, 100% ethanol three times for 30 minutes each at room temperature and xylene three times for 20 minutes each at room temperature. The tissue was then embedded in paraffin at 58 °C using an automated system. 10 µm thick sections were cut using a rotary microtome and the sections were floated in a 56 °C water bath and mounted onto gelatin-coated histological slides. The slides were left to dry overnight at room temperature. Slides with paraffin-embedded sections can be stored either at room temperature or in at 2-8 °C for several years in slide storage boxes.

2.5.5 Deparaffinisation and Rehydration

The sections were rehydrated with xylene twice for 10 minutes each time, 100% ethanol for 10 minutes, 95% for 10 minutes, 70% for 10 minutes, 5% ethanol for 5 minutes. Two further 10 minute washes in 0.01M PBS was performed to rehydrate the sections.

2.5.6 Optimisation of Antigen Retrieval

The methodology described below was finalised following the evaluation of various conditions for antigen retrieval to optimise staining. Prior to this, a many permutations of pH, heating method and timings were trialled. Antigen retrieval was assessed at 3 different pHs of citric acid and two heating methods as shown in Table. However, despite multiple attempts, the staining remained poor. Eventually, I was kindly given a protocol honed over the years by one of the postdoctoral fellow in the centre for neurosciences, Dr M Burgillos. This is what has been adapted with changes to the timings alone.

Table 2.3: Different variables tested for antigen retrieval

Water Bath 10 minutes	**Water Bath 35 minutes**	**Microwave 10 minutes**	**Microwave 35 minutes**
pH 6.0	pH 6.0	pH 6.0	pH 6.0
pH 8.1	pH 8.1	pH 8.1	pH 8.1
pH 9.0	pH 9.0	pH 9.0	pH 9.0

2.5.7 Antigen Retrieval

The slides were placed in a plastic coupling jar with antigen unmasking solution (Vector Lab, Cat No. H-3300) into pre-heated saucepan of water pre-heated to 90^{o}C. The slides were heated for 40 minutes, and then placed on the bench for 30 minutes to cool to room temperature. Slides were washed with 0.1M PBS three times for 10 minutes each time.

2.5.8 Staining

The tissues were demarked with a barrier pen and incubated in blocking solution for 1 hour at room temperature. The blocking solution consisted of 0.2% Triton + 5% normal donkey serum diltuted in PBS. Following this, the slides were incubated in primary antibody overnight. The slides were washed 3 times for 15 minutes each in wash buffer and incubated in secondary antibody for 2 hours. The slides were mounted with Vectaschield mounting medium with DAPI (Catalogue No. H-1200).

2.5.9 Immunohistochemistry Negative Control

Control experiments were carried out following protocol optimisation to gather information regarding background staining of cardiac tissue. Paraffin embedded sections of sham and TH cardiac tissue was processed using the above methodology with the omission of the primary antibody. The sections were incubated with the secondary antibody for 2 hours. There was no background staining of these slides.

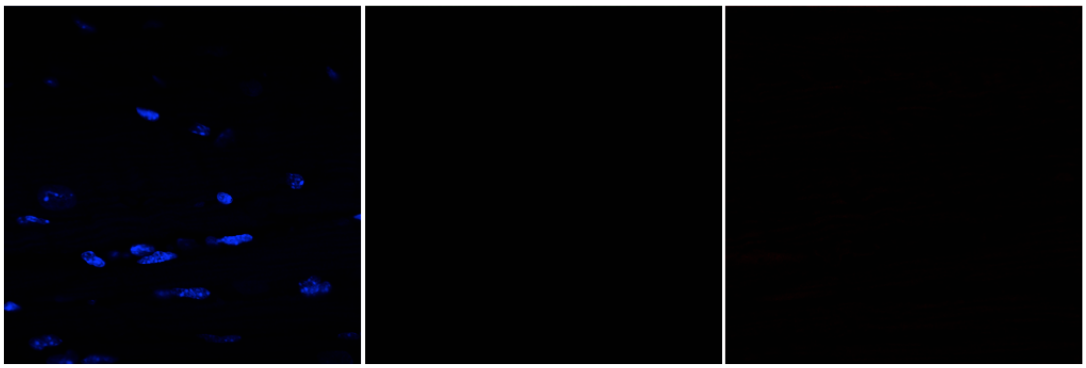


Figure 2.2: *Paraffin embedded cardiac tissue sections from sham-operated animals stained with DAPI (left) and chromogenic secondary antibodies AF488 (centre) and AF594 (Right) in the absence of a primary antibody*

2.5.10 Visualisation

Zeiss LSM710 confocal microscope was used for the imaging and processed on Zen imaging software. Image-J image processing programme was used to calculate pixel statistics by converting the single colour images to gray-scale. The mean pixel value was taken as a derivative of expression. Co-localisation was calculated using Zen imaging software.

2.6 Western Blotting

2.6.1 Introduction

In molecular biology, western blotting is used to separate and identify proteins based on their molecular size. Unlike, ELISAs, more than one protein can be identified at a time by re-probing the same membrane after stripping. However, unlike ELISAs, large number of samples can't be run at once due to the time intensive nature of the technique. Proteins extracted from tissue or cells are run through a porous polyacrylamide gel to separate them by size. The gels are loaded into an electrical chamber with positively and negatively charged electrodes, filled with running buffer. The negatively charged proteins are driven downwards towards the positive electrode on the application of a set voltage. The proteins are transferred from the gel to a nylon membrane by application of an electrical field perpendicular to the gel, this causes the negatively charged protein to move away towards the anode and that leads it to the membrane. The membrane is probed

with antibodies of the protein of interest, followed by a secondary antibody attached to a chemiluminescent substrate to allow for visualisation.

2.6.2 RIPA buffer for protein extraction

RIPA (Radio immune precipitation assay) buffer consists of

50ml of 50 mM TrisHCl (pH 7.4)

8.76 grams of 150 mM NaCl

10 ml of 1% Triton X-100

5g 0.5% Sodium deoxylcholate

1g 0.1% SDS

2ml 1 mM EDTA (0.5 M stock),

0.42g of 10 mM NaF

ddH2O to 1000 ml

Mixture of these reagents was then divided into 50 ml aliquots and cocktail of protease inhibitor and phosphatase inhibitor tablets were added (Pierce™ Protease and Phosphatase Inhibitor Mini Tablets Cat no88668).

2.6.3 Protein extraction

A portion of the heart was excised and weighed. A millilitre of RIPA buffer was added to every 0.1g of tissue.The tissue was placed on glass slide in RIPA over dry ice and gently minced using a fresh razor blade. Then the minced tissue was further disrupted in Dounce homogeniser with 20 strokes on ice. The sample was then sonicated on ice and centrifuged at 13 000 rpm for 20 minutes at 4^{o}C. The supernatant protein was collected and stirred in -80 for further analysis.

2.6.4 Mitochondrial separation

A mitochondrial isolation kit (Abcam) was used following the method as described by manufacturers. All reagents were provided in the kit.

1. 0.2-0.4 g of cardiac tissue was washed with 1.5 ml of wash buffer.
2. The tissue was then placed in the pre-chilled dounce homogeniser and disrupted by 30-40 strokes.
3. The homogenate was transferred to 2ml microtubes and each tube filled to 2 ml with isolation buffer.
4. The homogenate was centrifuged at 1000g for 10 minutes at 4^{o}C.
5. The supernatant was transferred in to a new tube and the pellet containing all large organelles was discarded.
6. The supernatant was topped up to 2ml with the isolation buffer and centrifuged at high speed at 12,000g for 15 minutes at 4^{o}C.
7. The supernatant was transferred to a new tube.
8. Pellets were suspended in 500 µl of isolation buffer with protease inhibitor cocktail
9. Aliquots were frozen -80^{o}C

2.6.5 Protein Quantification

Protein extraction from the hearts of the trauma haemorrhage models was quantified using a Bradford assay. Known amounts of bovine serum albumin were loaded alongside protein extraction samples from the trauma models. The protein concentration of the samples were then extrapolated for those samples to determine quantity to be loaded for the protein gels. The calculated amounts were loaded on to the gel were then transferred to a blot for protein expression analysis.

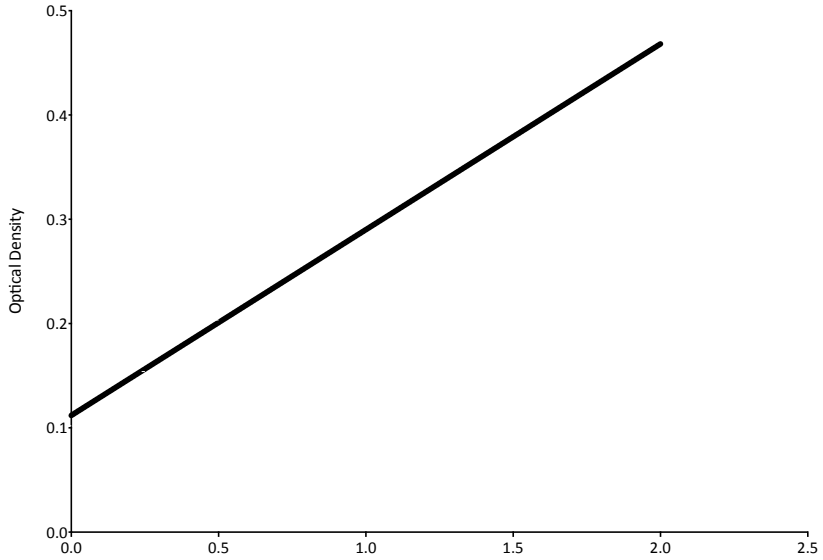


***Figure 2.3**: Bradford assay standard curve utilised for extrapolation of protein content in tissue samples*

A silver stain was performed on a gel run with the amount calculated from the Bradford assay. The silver stain that attaches to protein molecules in the sample allows determination of protein concentration in the tissue lysate. The silver stain is captured on a camera to allow quantification through optical density using the gel pro analyser. Image J quantifies the intensity of the optical density of each column. The optical density correlates to the concentration of protein. This was then used to standardise proteins of interests. This was repeated for the mitochondrial separation of the tissue lysates.

The optical density from each sample was used to determine how much of the cell lysate was to be loaded to determine protein concentration.

2.6.6 Gel Electrophoresis

1. NuPAGE® MOPS SDS Running Buffer (20X)-NP0001 was made up and tank filled

2. Bolt™ 4-12%, (10-well NW04122BOX) Bis-Tris Plus Gel was inserted into tank.
3. 10ul of samples were loaded with protein ladder (RPN800E-GE health care) into 10 well NuPage Bis-Tris Mini gel

2.6.7 Transfer of proteins from gel to nylon membrane

1. PVDF (poly vinyl di fluoride) nylon memebrane (Thermo fisher 88518) was activated in methanol before transfer into transfer buffer (Thermo fisher 1-Step transfer buffer-Cat no 84742)
2. Filter papers and Gel were soaked into transfer buffer
3. Gel was placed between transfer paper and blotting paper as a sandwich into Pierce power blotter. The protein transfer protocol for appropriate molecular weight was carried out as designated by the power blotter.

2.6 Electron Microscopy

Electron microscopy (EM) enables visualisation of tissue to 0.2nm scale. This uniquely allows for the study of ultrafine sub cellular structures in a great detail. Using EM proteins of interest can be visualised using antibody labelling with metal particles. Mice hearts were collected and immediately fixed in Glutaraldehyde. The samples were further fixed and processed for imaging by Dr Guilia Mastroianni, School of Biological and Chemical Sciences, QMUL.

2.6.1 Cacodylate buffer 0.1 M pH 7.4

Sodium cacodylate buffer [Na(CH3)2 AsO2 • 3H2O] was introduced for EM applications by Sabatini *et al.* in 1963 (114). The buffer acts to maintain a pH 5.0–7.4 and in doing so negates the need to add additional phosphates to sample

preparations. Mitochondria and other organelles can be damaged when exposed to the high concentrations of phosphates present in the traditionally used Sørensen's buffers. Cacodylate does not react with aldehyde fixatives as will amine-containing buffers (*e.g.,* Tris). Its efficacy in fixation solutions may be a result of the metabolism-inhibiting effect of the arsenate rather than any special buffering capacity.

2.6.2 Preparation of Buffered 2% Glutaraldehyde

A stock solution (A) of 0.2 M Sodium Cacodylate was made by dissolving 4.48 g sodium cacodylate in 100 mL distilled water and the acid stock solution of 0.2 M HCl (B) is made by diluting 20ml of 1M HCl with 80ml of distilled water. Preparation of the buffer solution: mix 100 mL of *stock A* and 5.4 mL *stock B* followed by the addition of distilled water to give a final volume of 200 mL. Then 15mL of 2% Glutaraldehyde is freshly constituted for every sample. 1.2ml of 25% glutaraldehyde is added to 13.8ml of cacodylate buffer**.**

2.6.3 Preparation of Osmium Tetroxide

4% aqueous solution of osmium tetroxide is diluted to 1% with ddH_2O of the appropriate volume.

2.6.4 Preparation of Araldite Resin

1. in a disposable polythene beaker, 40 ml of araldite CY212, 60 ml of dodecenyl succinic anhydride, 2.0 ml of methyl nadic anhydride and 1.0 ml of benzyl dimethylamine were combined in that order.

2. The mixture is stirred for at least 5 minutes, ensuring as little air as possible is incorporated into the mixture.
3. The mixture is allowed to stand for 1 hour to allow any air to escape.

2.6.5 Processing of Tissues

1. The tissue was dissected and fixed in 2% glutaraldehyde for 30 minutes at room temperature. The specimen is then cut into small 1 mm^3 and stored in fresh fixative in 4^oC for 1-7 days.
2. The specimen was removed and washed for 5 minutes in the cacodylate buffer. This is repeated three times.
3. The specimens are removed from the wash and incubated in 1% osmium tetroxide in buffer for 2 hours at 4^oC.
4. Wash in distilled water 3 x 5 minutes.
5. Dehydration steps in 25% acetone for 15min, 50% for 15min, 75% for 15 minutes and 100% twice for 20 minutes.
6. The samples were impregnated in increasing concentration of Araldite in acetone, 25% for 30 minutes, 50% for 30 minutes, 75% for 30 minutes and 100% for 1 h three times.
7. Following this, the sample was stored in fresh Araldite for 24-72 hours
8. Samples are stored at 48 hours for 60°C.
9. Thin sections were cut with a glass knife at a Reichert Ultracut E microtome and collected on uncoated, 300 mesh copper grids.
10. High contrast was obtained by post staining with saturated aqueous uranyl acetate and lead citrate for 4 min each (115).
11. The grids were examined in a JOEL JEM-1230 transmission electron microscope at an accelerating potential of 80 kV.

2.7 Statistical analysis

Statistical analysis was performed using IBM SPSS v22 and GraphPad Prism was

used for graph generation. Non-parametric comparisons of groups were performed using Mann-Whitney U and Kruskal-Wallis tests. Significance of categorical data was determined using Fisher's exact test. Linear relationships were determined using Spearman's rank correlation coefficient. Multivariate analysis was also performed using variables with a univariate correlation with a statistical significance of $p<0.1$. Overall, $p<0.05$ was taken to be statistically significant.

Chapter 3

Trauma Associated Cardiac Injury and Dysfunction (TACID)

A Clinical Study

3.1 Introduction

Several studies have retrospectively investigated the presence of cardiac injury in trauma patients. The incidences of cardiac events and rise in cardiac biomarkers have been correlated to pre-existing cardiac disease as well as the presence of direct cardiac injury. The Trauma Associated Cardiac Injury and Dysfunction (TACID) study was designed as a prospective observational study to simultaneously look at these different contributory factors of cardiac injury in trauma as well as their effect on clinical outcomes and biomarkers.

Cardiac disturbances after severe injury remain poorly characterized and the underlying mechanisms remain unclear/unknown. Deaths from fulminant multiple organ failure are often associated with inadequate cardiac output and increasing requirements for inotropic support(116,117). Trauma patients have been repeatedly demonstrated been shown to develop rhythm disturbances and to suffer from ischemic cardiac events(26). This Trauma-Induced Secondary Cardiac Injury (TISCI) may contribute to the late mortality and long-term morbidity after trauma(118).

It has been previously shown that patients who develop adverse cardiac events (ACEs) have elevations of plasma cardiac injury biomarkers very early in their clinical course(118). Heart-specific Fatty Acid Binding Protein (h-FABP) in particular was elevated immediately on admission and correlated with the development of ACEs

and subsequent mortality(118). In this small retrospective study, it appeared that the development of TISCI appeared to be strongly related to the degree of shock on admission, and there was a suggestion that the presence of cardiovascular disease prior to traumatic injury may also be a contributing factor (118).

The overall objective of this study was to investigate the drivers of TISCI and further understand the risk factors and outcomes associated with this condition. Our first aim was to determine the incidence of ACEs and their effect on outcomes in a prospective cohort of trauma patients. Our second aim was to determine the association of elevated admission levels of cardiac injury markers with the subsequent development of ACEs, and to determine the predictive power of these biomarkers.

3.2 Results

3.2.1 Patient characteristics

TACID was conducted at Royal London Hospital, a level I trauma centre in London from September 2010 to October 2012. All patients who were above the age of 16 and met the criteria for trauma team activation were screened for inclusion. The completion of the study was marked by the recruitment of 300 patients in October 2012. Following attempts to seek consent, 10 patients were excluded as they did not want to be included in the study.

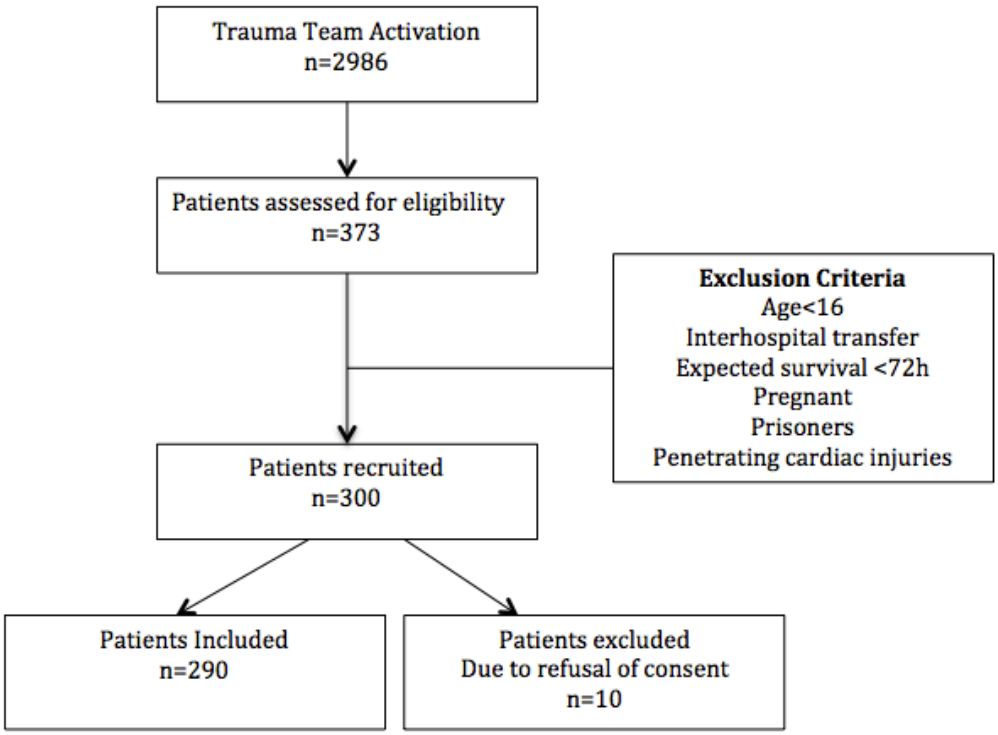


Figure 3.1 Patient recruitment flowchart for TACID

The study population had a median age of 38 years and 73% male. The majority of the patients, 82% had suffered a blunt injury, (82%) and with a median injury severity score (ISS) of 20. Despite the high injury severity score, this was not reflected by clinical parameters of shock on admission, with a median systolic blood pressure (SBP) of 125mmHg and lactate of 1.9mmol.

3.2.2 Adverse Cardiac Events

The development of adverse cardiac events was the primary endpoint of this study. Adverse cardiac events were diagnosed using ECGs and defined as arrhythmias requiring intervention, ischaemic ECG changes such as T wave inversion, ST elevation or depression and new onset bundle branch block. The diagnoses were

made by the clinician's independent to the research team using a combination of clinical evidence; ECGs, troponin changes and requirement for intervention.

During their admission of the 290 patients recruited, 38 developed ACEs. The study population was then dichotomised according to the presence of ACE. Although not statistically significant, the ACE cohort had a higher median age of 47 years compared to 37 years in the no ACE group. Notably 29% of the patients in the ACE group were less than 30 years old. The ACE group also had a higher percentage of male patients. However neither of these differences was of statistical significance.

The ACE cohort was more severely injured with higher ISS median of 30 in comparison to 13 in the no ACE group. The ACE group was more hypotensive than the no ACE group with a median SBP of 100 vs. 130 and more shocked with a lactate of 3.5 vs. 2.0 respectively.

Table 3.1: Patient demographics and injury characteristics

	All patients	No ACE	ACE	Univariate Analysis
No Patients (%)	290	252 (87%)	38 (13%)	**<0.01**
Demographics				
Median Age (years)	37 (16-91)	37 (26-51%)	47 (30-59)	**0.05**
<30	102 (35%)	91 (36%)	11 (29%)	
31-45	86 (30%)	78 (31%)	8 (21%)	
46-65	71 (24%)	61 (24%)	10 (26%)	
66-80	22 (8%)	16 (6%)	6 (16%)	
>81	9 (3%)	6 (2%)	3 (8%)	
Male	211 (73%)	214 (85%)	27 (71%)	0.07
Time To Sampling	92 (71- 115)	91 (73- 110)	100 (76- 116)	
Injury Characteristics				
Blunt	237 (82)	206 (82)	31 (82)	
ISS	17 (9-29)	13 (9-25)	30 (25- 43)	**<0.01**
Thoracic AIS	3 (0-3)	1 (0-3)	3 (2-4)	**<0.01**
H&N AIS	0 (0-3)	0 (0-3)	3 (0-4)	**0.01**
Admission SBP	126 (107- 146)	130 (114-147)	100 (67-125)	**<0.01**
Admission Lactate	2 (1.3-3.4)	2 (1.3-3.1)	3.2 (1.3-6.4)	**0.03**

Comparison of patient characteristics between the two cohorts. Median values are given for all variables with interquartile ranges in paranthesis except for sex, number of patients in each age category and blunt injuries which is shown as the number of male patients in the cohort with percentage in parenthesis. The p values were obtained from mann-whitney u analysis between the groups.

Direct thoracic injury has been shown to be associated with cardiac injury and a rise in cardiac biomarkers. In addition the patients who developed ACE had more significant thoracic (AIS score 3 vs. 1) and head injuries (AIS 0 vs 3). Patients with severe thoracic injury were more likely to develop an adverse cardiac event (42% vs

18%, p=0.01). However, severe head injury (AIS>3) was not associated with a higher prevalence of cardiac events.

Table 3.2: Prevalence of ACE in severe head and thoracic injury

	No ACE (n=252)	ACE (n=38)	p value
Severe thoracic injury (AIS >3)	45 (18%)	16 (42%)	**0.01**
Severe Head injury (AIS >3)	51 (20%)	12 (32%)	0.09

Values are given as n values with percentages in parenthesis. The p values were obtained from mann-whitney u analysis between the groups.

3.2.3 Pre-injury factors

Following recruitment, the patient's past medical history and medication history were obtained from the patients and their relatives. These were confirmed with the medical history obtained from their general practitioner's records. Overall, 7% of patients had a pre-existing diagnosis of ischaemic heart disease. Furthermore, 6% had a diagnoses of diabetes mellitus, 11% Hypertension, 8% Hypercholesterolaemia, 1% cerebrovascular accidents and 1 patient had peripheral vascular disease. All these pathologies could be indicative of potential, undiagnosed arterial disease.

On comparison of pre-injury diagnoses of cardiovascular disease between the ACE and no ACE cohorts, although the prevalence of IHD was comparable, the rates of hypertension (29% vs 8%) and hypercholesterolaemia (24% vs 6%) were significantly higher in the ACE group. Patients who developed adverse cardiac events were

more likely to have been using calcium channel blockers and statins prior to their injury. This may be reflective of the increased incidence of hypertension and hypercholesterolaemia in this group rather than the medications being causative factors.

Table 3.3. Patient demographics and injury characteristics in ACE and no ACE groups

	All patients	No ACE	ACE	P value
No Patients	290	252	38	
Cardiovascular disease				
IHD	20 (7%)	14 (6%)	5 (13%)	0.13
Diabetes	16 (6%)	11 (4%)	4 (11%)	0.18
Hypertension	32 (11%)	20 (8%)	11 (29%)	**0.02**
Hypercholesterolemia	24 (8%)	14 (6%)	9 (24%)	**0.02**
PVD	1 (<1%)	0	1 (3%)	0.15
CVA	3 (1%)	3 (1%)	0	0.61
Medication				
Anti-Platelet	10 (3%)	6 (2%)	4 (11%)	**0.03**
Oral Hypoglycaemic	7 (2%)	5 (2%)	2 (5%)	0.23
Insulin	3 (1%)	1 (<1%)	2 (5%)	**0.05**
Anticoagulants	4 (1%)	2 (1%)	2 (5%)	0.08
Beta blocker	6 (2%)	4 (2%)	2 (5%)	0.18
ACEi	16 (6%)	12 (5%)	4 (11%)	0.14
Ca Channel blocker	9 (3%)	4 (2%)	5 (13%)	**0.03**
Diuretic	8 (3%)	6(2%)	2 (5%)	0.28
Anti anginal	1 (<1%)	1 (<1%)	0	0.87
Nitrate	3 (1%)	3 (1%)	0	0.66
Statin	18 (6%)	10 (4%)	8 (21%)	**<0.01**

Comparison of distribution of pre-injury cardiovascular diagnoses and medication between the two cohorts. All values are given as n values with the value as a percentage of total number in the individual cohort in parenthesis. The p values were obtained from mann-whitney u analysis between the groups.

3.2.4 Electrocardiograms

Electrocardiograms (ECGs) are a familiar and easily accessible examination tool, which are in fact part of the ATLS guidelines for the primary survey of the injured patient. In routine practice however, due to the cumbersome nature of the equipment and the time critical environment they are often not completed. Patients included in the study were protocoled to have ECGs alongside blood sampling on admission, at 24 and 72 hours. Although 94% patients had ECGs on admission due to direct intervention by the research fellows, only 56% of patients had ECGs at 24 hours and 83% at 72hours. Nine (3%) patients did not have any ECGs at any time point.

The ECGs were studied by three physicians; two clinical research fellows (more than 3 years post qualification from medical school, and independent of the study) and one independent medical registrar (5 years post qualification from medical with postgraduate exams in general medicine).

Table 3.4: ECG changes on admission, 24 hours and 72 hours.

ECG Changes	Number of Patients		
	Admission n (% of total= 272)	**At 24 hours n (% of total=148)**	**At 72 hours n (% total= 109)**
T wave inversion	16 (6)	19 (13)	12 (11)
RBBB	9 (3)	3 (2)	3 (3)
Sinus Tachycardia	8 (3)	0	1 (1)
ST changes	7 (3)	4 (3)	2 (2)
AF	6 (2)	1 (1)	3 (3)
LBBB	4 (1)	1 (1)	0
Prolonged QT	3 (1)	1 (1)	0
LAD	2 (1)	1 (1)	1 (1)
1st degree heart block	2 (1)	1 (1)	1 (1)
Ventricular Ectopics	2 (1)	0	2 (2)
RAD	1 (<1)	3 (2)	1 (1)
Sinus Arrhythmia	0	1 (1)	0
Sinus Bradycardia	0	0	1 (1)

Changes have been divided into changes seen T wave inversion documented if noted on 2 leads or more, right bundle branch block (RBBB), Sinus Tachycardia (HT>110bpm), ST segment changes, atrial fibrillation (AF), left bundle branch block (LBBB), Prolonged QT time, left axis deviation (LAD), Right axis deviation (RAD)

Fifty-four patients had ECG changes on admission, with T wave inversion being the most common ECG change with a prevalence of 6%. These were most frequently (63%) seen in the chest leads. Right bundle branch block (RBBB), sinus tachycardia and ST changes were also noted. Two of the nine patients who had RBBB had significant thoracic injury with a thoracic AIS of 4 and 5 with lung injury. The other

seven had a thoracic AIS of 1 or less. Of those seven patients, one had persistent RBBB, noted on admission and at 24 and 72 hours. Whilst it was transient in nature in the other six patients noted only at one point, with 1 patient developing RBBB at 24 hours.

At 24 hours, ST changes became marginally more prevalent, seen in 3 % of patients, all were new at 24 hours and transient. Two of the four patients had significant thoracic injury with an AIS of 3 and 5.

Sinus tachycardia, atrial fibrillation and Left BBB (LBBB) were also commonly transient changes at admission. The initial changes are likely to be multifactorial and individual to the patient due to a combination of direct cardiac injury, rate related changes, hypoperfusion, adrenergic stimulation and underlying vessel disease.

Furthermore, in this cohort of 290 patients, of the 38 (13%) patients who developed adverse cardiac complications, 15 (5%) patients had ischaemic changes on their ECGs, of whom 6 (2%) had one or more cardiac risk factors. In most settings, these patients would have warranted further investigation and cardiology follow up. However, only one of those 6 patients went on to have angiography. These changes are often attributed to high heart rates and therefore often forgotten when patient recovers from the initial resuscitative period. However, the manifestation of these

ischaemic changes may signify underlying arterial disease and especially in those with pre-disposing risk factors validate further investigation.

3.2.5 Cardiac biomarkers in trauma patients

Blood samples were taken from patients on admission, 24 hours after injury and 72 hours after injury were centrifuged and the plasma stored in -80^{o}C prior to processing. All admissions samples from admission were processed for troponin, nt-ProBNP and h-FABP. The Troponin ELISA completed for the admission samples did not show any differentiation between the samples. Following discussion with the manufacturers, it was decided not to further proceed with testing of the 24 hour and 72 hour samples.

All but one patient had blood sampling on admission with the bloods (table 3.1) taken at a median sampling time of 92 minutes overall, 91 minutes in the no ACE group and 100 minutes in the ACE group. The difference in time was not of statistical significance. (Table 3.1). 81% of patients had blood sampling at 24 hours and 62% at 72 hours. At 72 hours, patient refusal for blood sampling reduced protocol compliance as on discharge to the ward, arterial or central lines were removed necessitating venepuncture for purely research purposes.

On admission, the patients who develop ACEs had plasma h-FABP levels that were four folds higher than the patients who did not develop ACE. At 24 hours and 72 hours there was no significant difference. The median plasma levels of nt-ProBNP in the patients who did not develop ACE was marginally higher than the normal reference value provided by the manufacturers. It was also higher than the median

value of nt-ProBNP in the no ACE group, however this was not statistically significant.

Table 3.5: Changes in cardiac biomarkers in the ACE group and no ACE group on admission, at 24 hours and 72 hours

	No ACE	**ACE**	**P values**
h-FABP (ng/ml)			
Admission	15.2 (5.3- 50.7)	62.0 (26.3 – 110.6)	<0.001
24 hour	10.8 (3.5- 33.9)	53.7 (8.2- 129.6)	0.001
72 hours	4.4 (1.7- 9.6)	8.9 (2.5- 54.5)	0.04
nt-ProBNP **(pmol/l)**			
Admission	400.6 (228.0 – 651.0)	363.6 (0-473.5	0.26
24 hour	422.8 (116.8- 994.9)	461.5 (226.9- 1154.6)	0.73
72 hours	246.3 (104.4- 447.6)	231.3 (116.3- 573.7)	0.73
Troponin			
Admission	0 (0-0)	0 (0-0.3)	0.299

Values given as medians with interquartile ranges are given in parenthesis. The p values were obtained from mann-whitney u analysis between the groups.

There was no difference in plasma cardiac biomarker levels between groups with and without severe thoracic injury. In the group with severe head injury, the patients had higher levels of plasma nt-ProBNP on admission (396.2 vs 465.0 pmol/l, p=0.03).

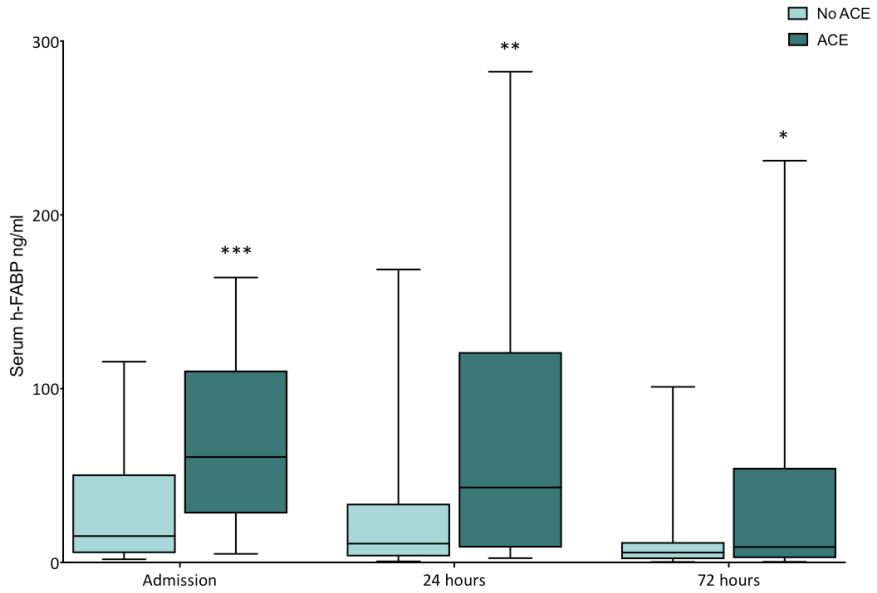


Figure 3.2*: Comparison of h-FABP levels in patients with and without ACE. On admission the ACE group has higher levels of 62 ng/ml vs. 15.2 ng/ml, p≤0.001 ***, at 24 hours- ACE 53.7 ng/ml vs 10.8 ng/ml in the no ACE group, p ≤0.01** and at 72 hours, the ACE group has h-FABP levels of 8.9 ng/ml vs 4.4 ng/ml in the no ACE group, p≤0.05*.*

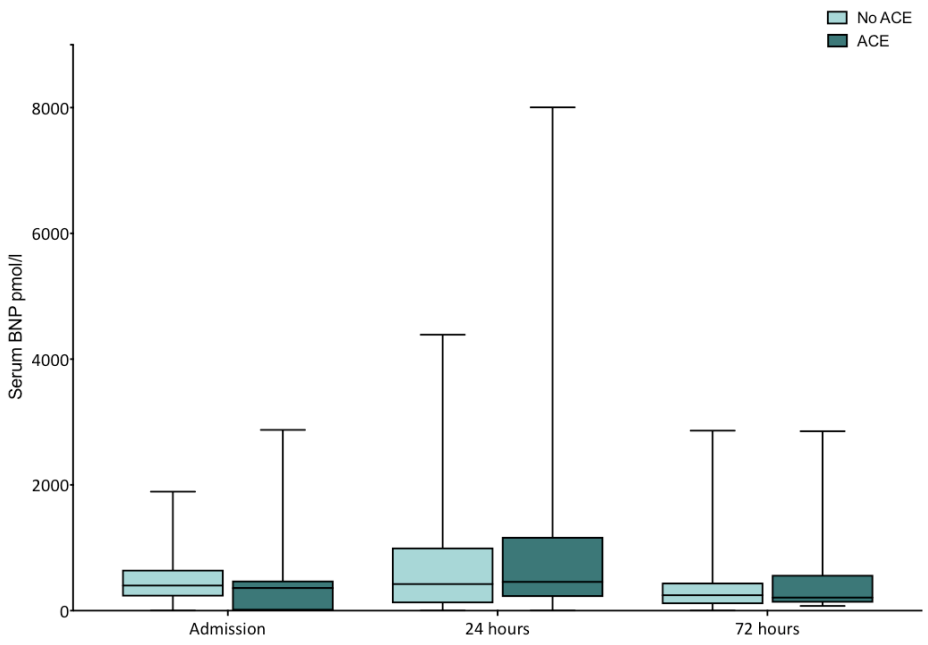


Figure 3.3: *Comparison of BNP levels in patients with and without ACE. On admission the ACE group had BNP levels of 363.6 vs. 400.6 (p=0.26), at 24 hours the ACE group had 461.5 pml/L vs. 422.8 pmol/L in the no ACE group, p=0.73 and at 72 hours the ACE group had 231.3 pmol.L vs. 246.3 pmol/L, p=0.73.*

Table 3.6: Cardiac biomarkers in mild (AIS<3) and severe (AIS>3) head and thoracic injury (AIS>3) on admission, at 24 hours and at 72 hours

	Mild Injury (AIS<3)	Severe Injury AIS>3	p value
Thoracic Injury			
HFABP			
Admission	20.6 (6.2- 62.9)	14.4 (5.4- 60.1)	0.33
24 hours	12.9 (4.5- 53.7)	18.4 (2.7- 37.9)	0.44
72 hours	5.9 (1.9- 12.3)	2.4 (0-12.0)	0.06
nt-ProBNP			
Admission	380.9 (215.7- 614.7)	435.2 (226.3- 706.4)	0.55
24 hours	422.8 (117.0- 905.7)	491.8 (137.4- 2169.5)	0.19
72 hours	242.8 (108.7- 484.1)	339.1 (95.7- 418.5)	0.86
Troponin			
Admission	0	0	0.81
Head Injury			
HFABP			
Admission	17.5 (6.2- 65.5)	22.5 (5.1- 49.8)	0.67
24 hours	15.6 (4.5- 48.4)	8.8 (3.2- 27.3)	0.13
72 hours	4.1 (1.7- 12.3)	6.4 (2.0- 28.1)	0.40
nt-ProBNP			
Admission	369.2 (208.7- 562.3)	465.0 (318.8- 846.4)	**0.03**
24 hours	448.0 (116.9- 1085.3)	418.2 (181.5- 964.7)	0.90
72 hours	231.4 (103.5- 417.0)	325.1 (112.3- 541.3)	0.41
Troponin			
Admission	0	0	0.16

The values are given as median with the interquartile ranges in parenthesis.

3.2.6 Echocardiograms

Echocardiograms were requested through the hospital cardiac physiology department at the Royal London on all patients. Due to the limited availability of the cardiac physiologist, only 43 (15%) patients had an echocardiogram, of those 65% had complete data sets with good views. In the patients who had echocardiograms, 19 (44%) patients had an adverse cardiac event during their admission. The left ventricular stroke volume, ejection fraction, fractional shortening, ventricular volumes and E:A ratios were all measured.

The stroke volume is the amount of blood expelled from the ventricle per beat and contributes to the cardiac output. This can be calculated by multiplying the velocity time integral by the left ventricular outflow tract (LVOT) area. Velocity time integral or the stroke distance is a measure of the distance the blood moves with each beat. Doppler signal from the left outflow tract is recorded and the sum of the individual velocities are computed and divided by time to give the velocity time integral. The diameter of the left ventricular outflow tract is measured in the parasternal long axis view and the area calculated using:

Stroke Volume*= LVOT area x VTI*

The left ventricular stroke volume for both the groups were at the lower end of the normal range and no different between the two groups (ACE group 67 ml vs. 66 ml no ACE).

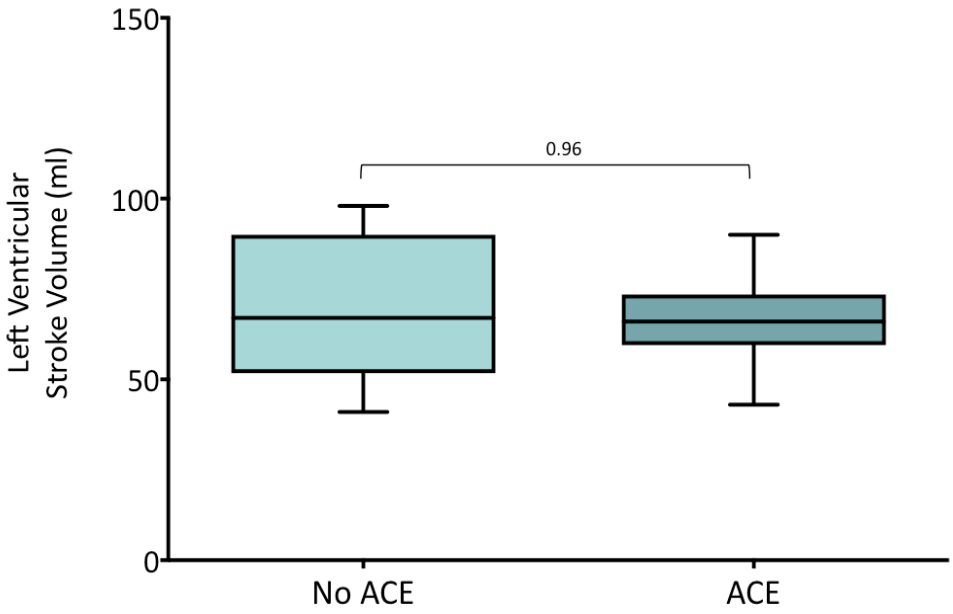


Figure 3.4: Comparison of left ventricular stroke volume between ACE (median 67ml- IQR 60-74ml) and no ACE group (median 66ml- IQR 52-90ml). The p value of 0.96 was obtained from mann-whitney u analysis between the groups.

End diastolic and end systolic volumes can be used to approximate preload, afterload and contractility. The end diastolic volume is the volume of a filled vemtricle. Therefore a decrease in preload due to reduced venous return would lead to a concurrent decrease in the end diastolic and systolic volume. Changes in ventricular contractility can also lead to changes in the ESV and EDV. Increase in heart contractility leads to a reduction in ESV and a concurrent reduction in EDV.

The left ventricular volumes are derived using the Teicholz formula to convert ventricular internal diameters during systole and diastole. The left ventricular internal diameter is calculated at end diastole and systole. The end systolic diameter is measured at the end of the t wave as seen on ECG, when the walls are at their most inward position. Similarly, the end diastolic measurement is taken at the beginning of the Q wave. Both measurements are taken along the apical long axis from the apex to the midpoint of the mitral plane on echocardiography in the four-chamber view and along the radial axis on a two-chamber view.

$$\boldsymbol{Left\ Ventricular\ Volume} = \frac{7}{(2.4 + LVID)}\ x\ LVID^3$$

The end systolic volume in the ACE group (29ml) was lower than in the no ACE group (38.5ml) but not of statistical significance. This is also reflected by the EDV with lower EDV in the ACE (median 99ml- IQR 86-109) group compared to the no ACE (median 109ml- IQR 90- 119) group. This may be due to the reduced venous pressure secondary to hypovolaemia in the ACE group or due to the increased myocardial contractility in a hyperdynamic, critically injured patient.

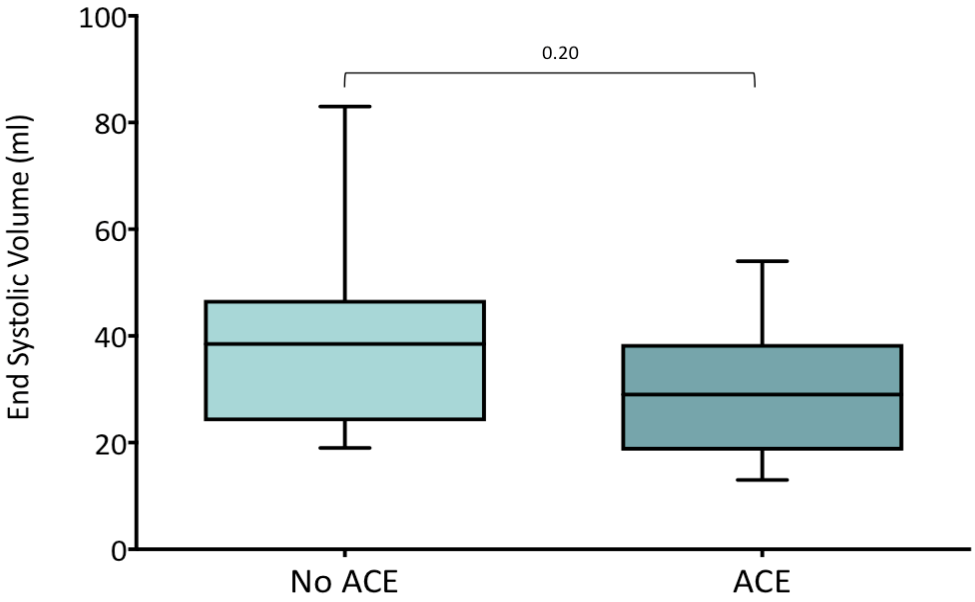


Figure 3.5: *Comparison of end systolic volume between ACE (median 29ml- IQR 18.5-38.5) and no ACE (median 38.5ml- IQR 24-47ml) group. The p value of 0.20 was obtained from mann-whitney u analysis between the groups.*

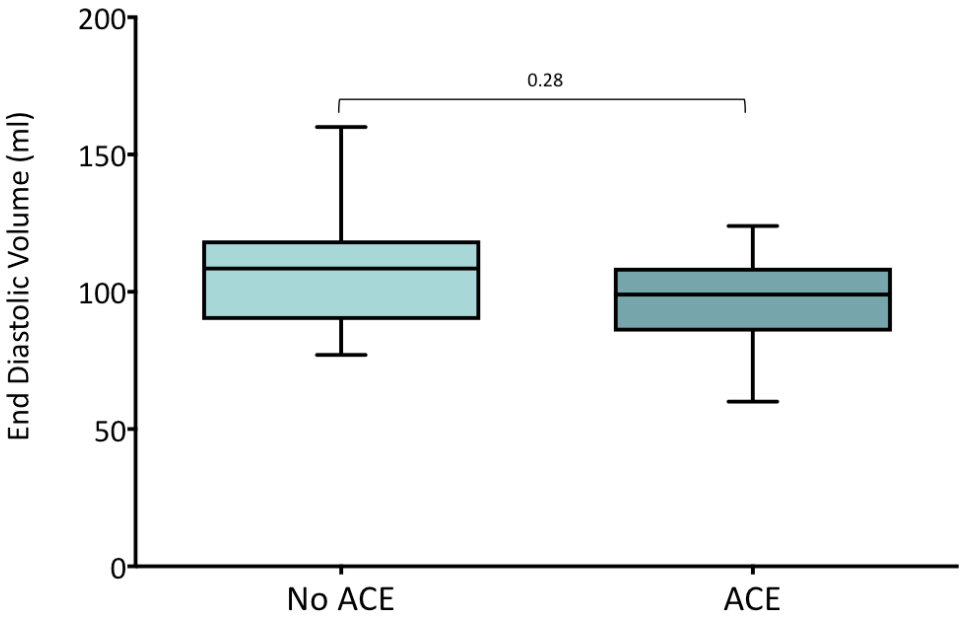


Figure 3.6: *Comparison of end diastolic volume between ACE (median 99ml- IQR 85.5- 109) and no ACE (median 108.5ml- IQR 90- 119) group. The p value of 0.28 was obtained from mann-whitney u analysis between the groups.*

Ejection fraction was used as a measure of left ventricular function. It is a derived ratio of the change in left ventricular volume over the cardiac cycle as compared to its maximal volume during diastole, as follows:

$$\boldsymbol{Ejection\ Fraction} = \frac{ED_{Vol} - ES_{Vol}}{ED_{Vol}}\ x\ 100$$

Surprisingly, despite the adverse cardiac events, the ejection fraction was not altered in the ACE group. The median ejection fraction was maintained at a normal level of 70% for both groups.

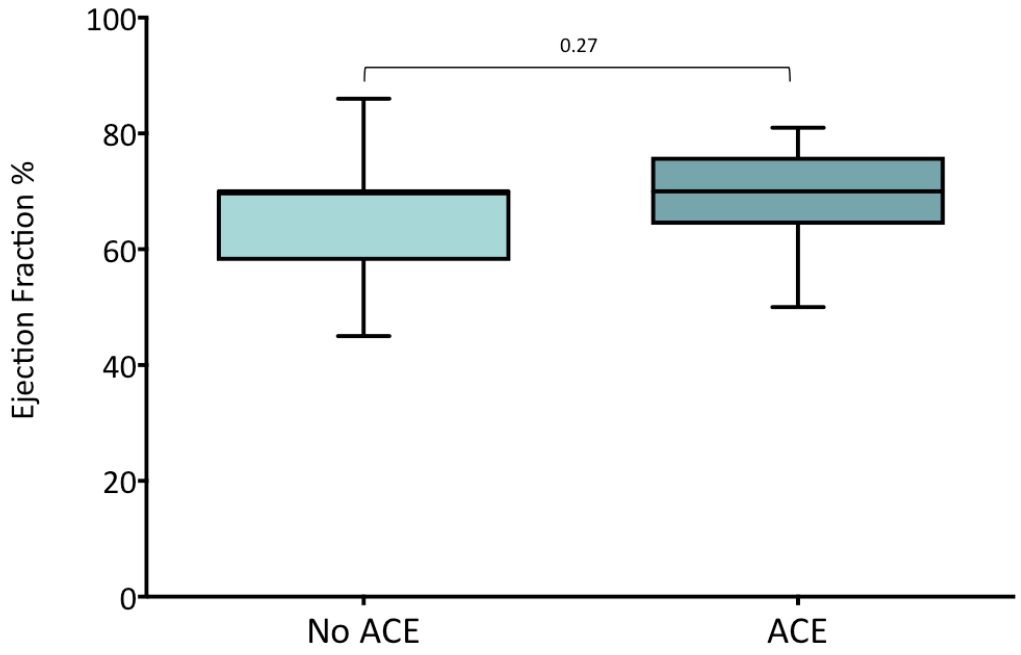


Figure 3.7: *Comparison of ejection fraction between ACE (median 70%- IQR 64- 76) and no ACE (IQR 70%- IQR 58-70) group. The p value of 0.27 was obtained from mann-whitney u analysis between the groups.*

Fractional shortening can be used as a measure of contractility. Measurements of end systolic diameter and end diastolic diameter are taken from the left ventricle. The ratio of the change in diameter over the cardiac cycle over the end diastolic diameter is calculated as:

$$\boldsymbol{Fractional\ shortening} = \frac{LVEDD - LVESD}{LVEDD}\ x\ 100$$

Fractional shortening seemed to have an upward trend with adverse cardiac events, rising to 38% compared to 32% in the no ACE group. Both values are within the normal range for fractional shortening.

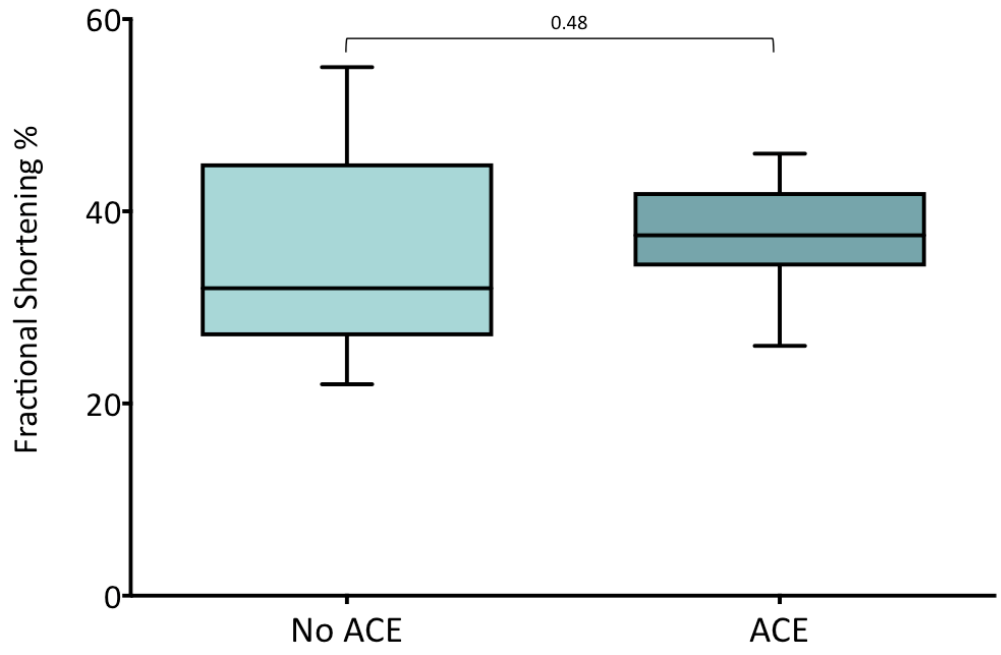


Figure 3.8: *Comparison of fractional shortening between ACE (median 38%- IQR 34-42) and no ACE (median 32%- IQR 27-45) group. The p value of 0.48 was obtained from mann-whitney u analysis between the groups.*

The interventricular septal end diastolic diameter (IVSDd) is measured in the parasternal view at the beginning of the Q wave. This can be used as a surrogate marker for left ventricular hypertrophy and ventricular mass. In the ACE group the IVSDd was 1.2cm compared to 1 cm in the no ACE group. This small difference may be due to this group having a greater number of hypertensive patients, however, both values are still within the normal limits.

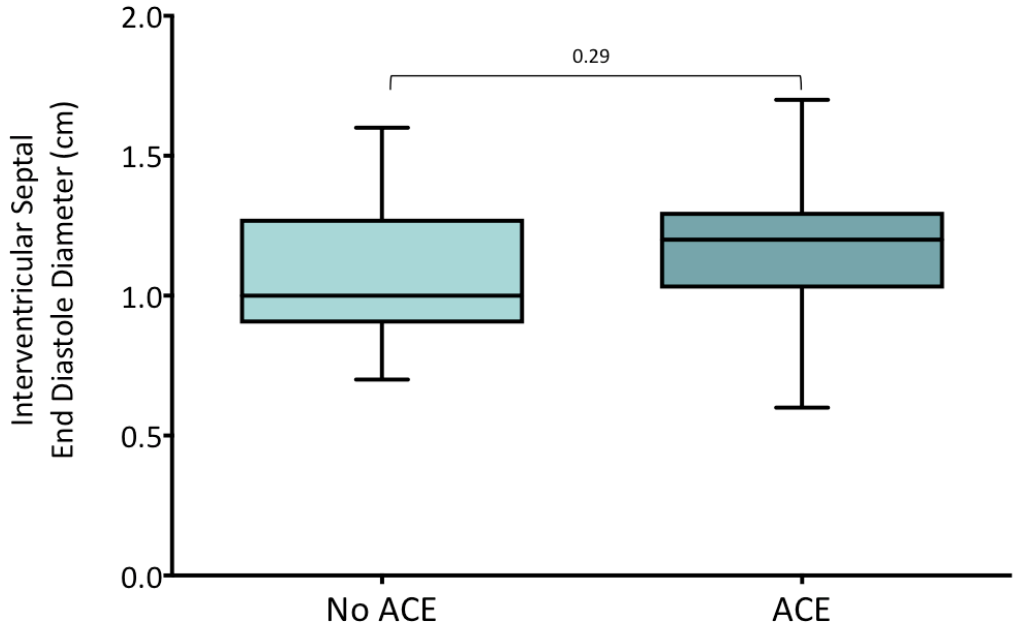


Figure 3.9: *Interventricular septal end diastole diameter in ACE (median 1.2cm- IQR 1.0- 1.3) and no ACE (median 1- IQR 0.9-1.3) group. The p value of 0.29 was obtained from mann-whitney u analysis between the groups.*

The E:A ventricular filling ratio can be used as a marker of diastolic function. The ratio takes into account the early diastolic (E) filling and late (A) filling of the ventricles. Normal E:A ratio varies between 1 and 2 with a reduction below 1 likely to represent diastolic dysfunction due to impaired relaxation or an increase in ratio above 2 can be due to restrictive diastolic dysfunction. In this cohort the median for both groups remain within normal limits, 1 in the No ACE group and 1.2 in the no ACE group with no statistically significant difference.

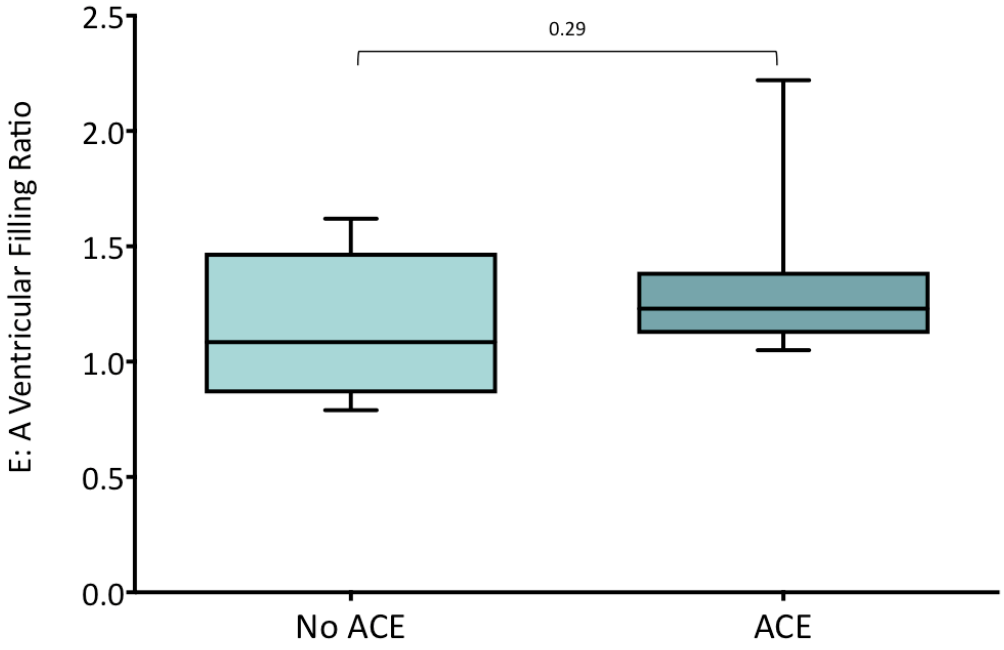


Figure 3.10: *E:A ratio in ACE (median 1- IQR1.12-1.39) and no ACE (median 1.2- IQR 0.9-1.47) group. The p value of 0.96 was obtained from mann-whitney u analysis between the groups.*

3.2.8 In- hospital Medications

During the patients' hospital admission, notes were reviewed and initiation of new medication documented by research fellows on a daily basis. A higher percentage of patients developing ACEs (37%) received Tranexamic acid compared to the group who did not develop ACEs (15%). Tranexamic acid administration coincides with the initiation of the major haemorrhage protocol. Therefore, this may be reflective of the increased severity of injury and potentially larger number of patients presenting with active bleeding. The higher rates of inotropic use, as well as diuretic use, in the ACE cohort could be suggestive of a state of shock. As previously mentioned in Section 3.2.4, 15 patients developed ischaemic changes, which would have warranted dual antiplatelet therapy in other patient groups. However, in the trauma patients, where bleeding is a concern, this is often not safe. Although not included in this graph- Aspirin alone was initiated in one of these 20 patients.

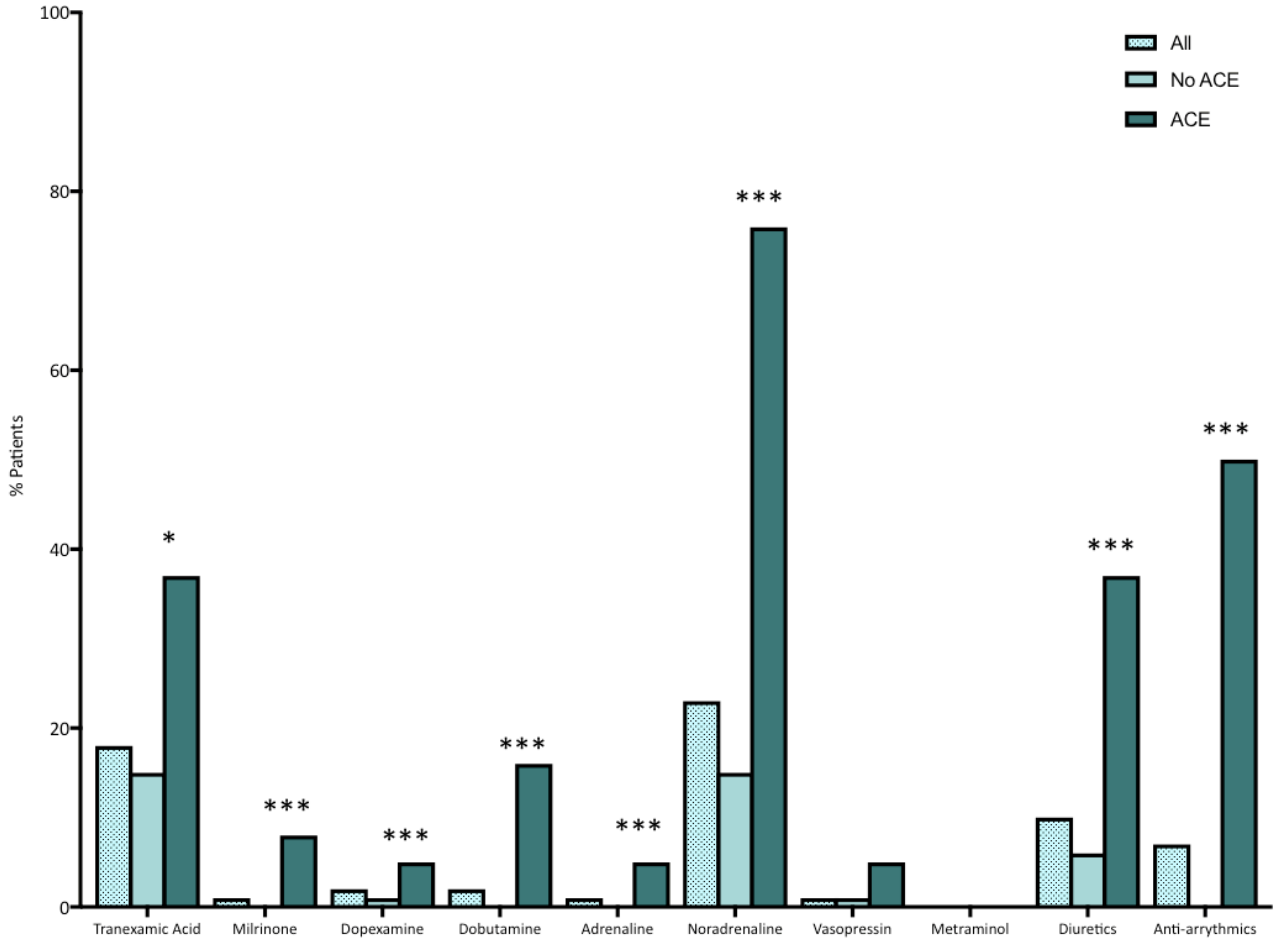


Figure 3.11*: Comparison of medications initiated in hospital between the groups with ACE group and without development of no ACE (* = p<group. (*≤0.05, *** = p<**≤0.01, ***≤0.001)*

3.2.9 Clinical Outcomes

The patients that developed ACEs were twice as more likely to require admission to ICU (95% vs 47%, p<0.01) and on average required 13 days longer in ICU when compared to those who did not develop ACE. In addition, those that developed ACEs spent 21 days longer in hospital (28 vs. 7 days, p<0.01). Cardiac events were also associated with four-fold increase in mortality (24% vs 6%, p<0.01).

Table 3.7: Comparison of outcomes

Outcomes	No ACE	ACE	p value
ICU admission (%)	47	95	**<0.01**
ICU Length of Stay (Days)	0 (0-2)	13 (6-19)	**<0.01**
Total Length of Stay (Days)	7 (2-15)	28 (14-47)	**<0.01**
Mortality Rate (%)	14 (5)	9 (3)	**<0.01**

ICU admission and mortality are given as n numbers with percentage of total patients in parenthesis. Median of the total length of stay is stated with interquartile ranges in parenthesis.

3.2.10 Predicting Adverse Cardiac Events

H-FABP was found to be associated with ACEs, which in turn was associated with longer critical care and hospital admission. Therefore, the use of h-FABP as a predictor of ACE was assessed using a ROC curve. The derived area under the curve (AUC) was 0.76 indicative of its positive predictive value.

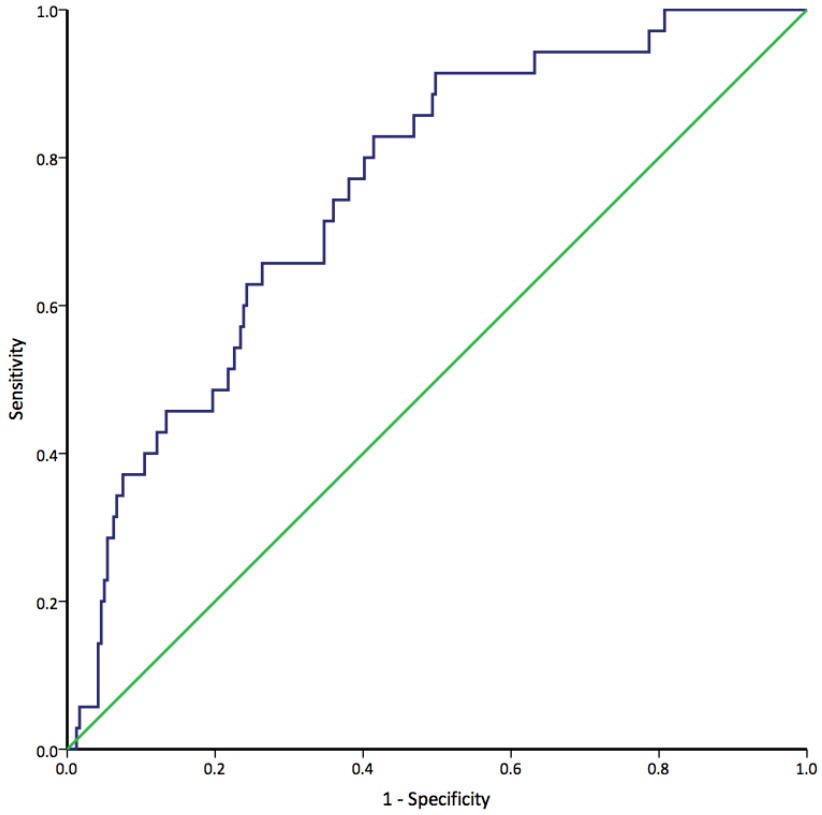


***Figure 3.12**: ROC curve of predictive strength of h-FABP for adverse cardiac event with AUC of 0.76*

A large meta- analysis study looking at h-FABP levels in myocardial ischaemia used 5 -16.8 ng/ml as diagnostic cut-off points. In this study population, 16.8 ng/ml has a sensitivity of 86% and specificity of 51% for predicting adverse cardiac events (119) Patients with plasma h-FABP levels above 16.8 ng/ml were assumed to have myocardial injury and were defined as having trauma induced secondary cardiac injury (TISCI). Outcomes of these patients were compared to those not developing TISCI.

Table 3.8: Comparison of patient characteristics in development of TISCI

	No TISCI H-FABP< 16.8ng/ml	**TISCI HFABP>16.8ng/ml**	**P value**
Demographics			
No Patients	129 (44%)	161 (56%)	
% developing an ACE	6 (5%)	32 (20%)	<0.001
Median Age	34 (23-46)	41 (28-59)	0.03
<30	52 (40%)	49 (30%)	
31-45	42 (33%)	43 (27%)	
46-65	25 (19%)	45 (28%)	
66-80	5 (4%)	17 (11%)	
>81	3 (2%)	6 (4%)	
Male	113 (88%)	125 (78%)	0.19
Cardiovascular Disease			
IHD	8 (6%)	12 (7%)	0.59
Diabetes	4 (3%)	11 (7%)	0.35
Hypertension	11 (9%)	21 (13%)	0.27
Hypercholesterolemia	8 (6%)	16 (10%)	0.49
PVD	0	1 (<1%)	0.58
CVA	1 (<1%)	2 (1%)	0.62
Injury Characteristics			
ISS	9 (4-16)	25 (13-34)	<0.001
Thoracic AIS	0 (0-1)	3 (1-4)	<0.001
H&N AIS	0 (0-2)	0 (0-4)	0.45
Admission SBP	137 (119-149)	120 (97-139)	<0.001
Admission Lactate	1.9 (1.3-3.0)	2.5 (1.6-4.3)	0.08

Demographics and cardiovascular diagnoses are given as absolute n values with percentages in parenthesis. Injury characteristics are stated as median values with interquartile ranges in parenthesis. (= p≤(*≤0.05, ** = p≤**≤0.01, ***≤0.001)*

Overall 161 (56%) patients had elevated h-FABP levels of >16.8 ng/ml and 32 (20%) of these subsequently developed an ACE (compared to 5% of those without TISCI, p<0.001, Table 1). Biomarker evidence of TISCI on admission was associated with a greater ISS and higher thoracic AIS. TISCI was also associated with lower admission blood pressures and higher lactate levels.

Table 3.9: Comparison of biomarkers and outcomes in TISCI

	No TISCI **H-FABP< 16.8ng/ml**	**TISCI** **HFABP>16.8ng/ml**	**P value**
Admission Cardiac Biomarkers			
H-FABP (ng/ml)	54.8 (35.9- 95.7)	51.3 (25.0- 88.8)	<0.01
nt-ProBNP (fmol/ml)	342.0 (257.6-631.6)	310.0 (254.9-522.1)	0.66
Troponin I (ng/ml)	0 (0-0)	0 (0-0)	0.65
Outcomes			
Inotrope days	0 (0-0)	0 (0-3)	
ICU Length Of Stay	0 (0-0)	1 (0-7)	<0.01
Total Length of Stay	4 (2-10)	15 (7-37)	<0.01
Mortality	6 (5%)	22 (14%)	0.05

Values are given as medians with interquartile ranges in parenthesis. (= p≤0.05, ** = p≤0.01)*

Patients with TISCI spent a median of 1 day longer on ICU and 11 days longer in hospital. TISCI was associated with a 3-fold higher mortality (14% vs 5%, p=0.01).

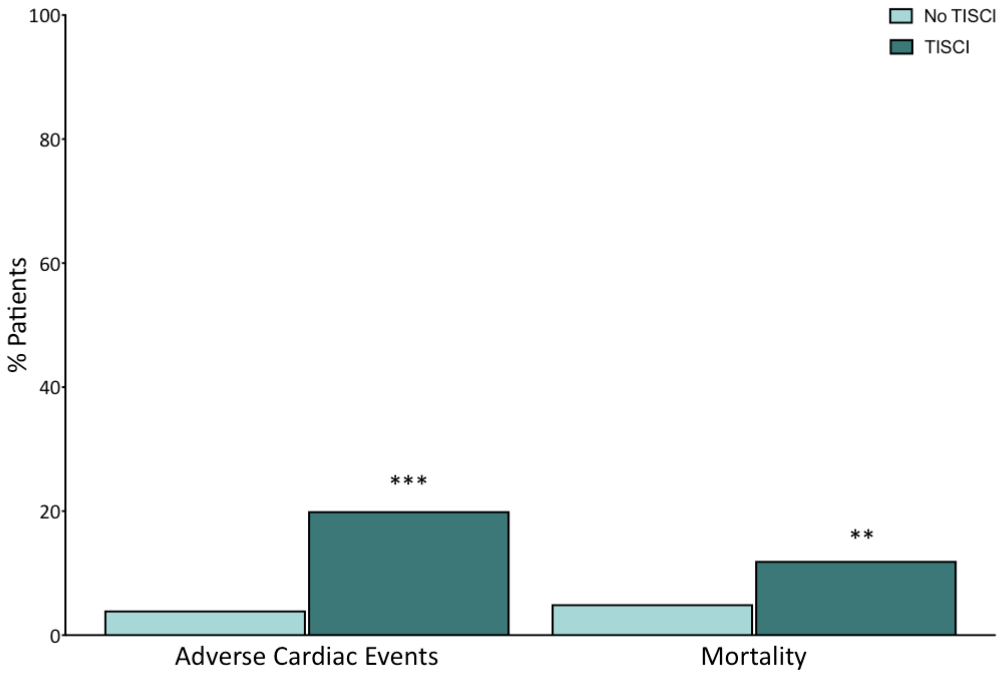


Figure 3.13*: Difference in adverse cardiac events 20% in those with TISCI vs 5% in those without (p≤0.001***) and 30 day mortality 14% in those with TISCI and 5% in those without (p=0.01)*

3.3 Summary of Chapter

This chapter presents the results of a prospective study of adverse cardiac events in critically injured patients, and explores the associative factors and the impact on outcome.

The 13% incidence of adverse cardiac events in this prospective study was similar to the previous smaller retrospective study presented by our group, but lower than that reported in other study populations (102,118,120). The commonest ECG changes was T wave inversion of which 63% occurred in 3 or more chest leads, followed by supraventricular tachycardias predominated, but there was also

evidence of ischemic injury. Susceptibility to the development of ACEs is identifiable on admission by levels of cardiac biomarkers, specifically h-FABP. While age, pre-existing cardiovascular diagnoses, and direct thoracic trauma appear to be risk factors for the development of TISCI, one third of patients had none of these features. The degree of shock was strongly associated with admission TISCI and the development of ACEs.

Patients with evidence of TISCI had double the baseline mortality while in those who went on to develop an ACE mortality was tripled. Although we primarily identified rhythm disturbances, it is not clear how, or if, these contributed to the mode of death. We did not specifically measure cardiac function but inotropic support was similar across the groups. There is some experimental evidence to suggest that myocardial damage occurs following trauma and haemorrhagic shock via a variety of postulated mechanisms (102,121,122). Human data on this is lacking, however, and it remains unclear how cardiac injury and dysfunction may contribute to increased morbidity and mortality in trauma patients.

The potential usefulness of three cardiac biomarkers in predicting ACE on admission in trauma patients was studied. On admission, h-FABP was the only marker that was significantly raised, and 56% of the patients had high levels of h-FABP suggestive of significant myocardial cell damage with leakage of cytoplasmic protein (123). Furthermore, the patients who developed ACE also had persistently higher h-FABP levels compared to the no ACE group. Although, h-FABP levels positively correlated with thoracic injury severity, a third of patients with myocardial leak had insignificant chest trauma. Global injury severity and the degree of shock appear to be strong factors in the development of TISCI. Putative mechanisms include ischemia, damage-induced inflammation or reperfusion injury (103,122,124,125). However, the specific cardiac pathology and underlying mechanisms have not been elucidated. Nevertheless, the presence of myocardial leak immediately on admission strongly correlated with adverse outcomes. H-FABP may be a useful early biomarker for patient risk stratification. In an inpatient setting, this could aid

clinical decisions regarding monitoring and nursing requirements and on discharge, delineate patients who may need ongoing cardiac surveillance.

There are several limitations to this study. Although prospectively conducted, we did not formally measure cardiac output or other aspects of cardiac function in the cohort. Although, attempts were made to obtain echocardiographic data on all patients, deficiencies in service provision and a lack of availability cardiac physiologists or clinicians competent in echocardiography lead to the failure to obtain sufficient data. Furthermore, the parameters measured in this study are all to different degrees volume and rate dependent. Newer techniques such as tissue doppler index and global longitudinal strain (GLS) as measured by speckle tracking are more reliable indicators of cardiac function (126). Tissue Doppler imaging can be used to measure the changes in myocardial velocity over a cardiac cycle with measurements taken at systole (S'), early diastolic relaxation (e') and during late diastole (a') (126). These show a linear correlation with LVEF but are also more sensitive markers of function as they are less volume dependent (127). Two-dimensional speckle tracking is an emerging technique for measuring cardiac contractility through analysis of the migration 2 pixels on 2-D clips. This has shown to correlate well with traditional measures of systolic and diastolic function and also more sensitive at identifying reduced function. It is also volume independent and unlike the tissue doppler measurements also angle independent (128). However, in a centre with limited echocardiography facility, it was not possible to ascertain these measurements. No other functional data was obtained in the form of dynamic cardiac output monitoring (lithium dilution, thermodilution, pulmonary artery catheters) or cardiac MRIs.

Further work is needed to monitor and image cardiac function in critically ill trauma patients. Data on the exact mode of death of these patients were not collected and therefore we were not able to link the adverse cardiac events to the mode of death of the patients, or to describe how cardiac dysfunction may have contributed. The patient group who develops cardiac injury is significantly more injured and more

shocked, therefore it may be inevitable that these patients go on to develop of secondary cardiac injury as part of the systemic inflammatory insult of polytrauma, leading to multi organ failure and eventually leading to death. Late deaths in trauma are often multifactorial, but more work needs to be done to explore the specific contribution TISCI makes to morbidity and mortality after trauma.

In this large prospective cohort study we have confirmed the presence of biomarker-identified TISCI on admission in major trauma patients. It carried a high risk of subsequent adverse cardiac events and poor outcomes.

Chapter 4

Catecholamines In

Trauma Induced Secondary Cardiac Injury

4.1 Introduction

The physiological stress of severe injury leads to a hyperadrenegic state. In 1975, Jaattela published data on 45 severely injured patients in whom it was noted that up to 6 hours post injury the catecholamine levels were greater than their baseline taken at 8 days post injury (129). This was also mirrored in studies in traumatic head injury, which induces with surge of catecholamines and unopposed sympathetic activation (130). Increased catecholamine levels have also been demonstrated in burns patients, which is furthermore associated with increased infection rates (131). Hyperadrenergic states with high levels of catecholamines can be deleterious to the heart due to excessive stimulation of the adrenergic receptors, particularly at the apex of the heart. Excessive adrenergic stimulation of cardiomyocytes induces rapid changes in calcium influx and even cardiomyocyte death (132).

In traumatic brain injury patients studies have shown cardiac dysfunction, with prevalence varying from 8% to 28% in different studies (133-135). Beta-blockade in TBI has been shown to reduce hospital mortality in nine observational studies (136). However, exposure to beta blockers was also associated with an increase in infection rates and length of hospital stay (97) (137,138).

In polytrauma patients, high circulating levels of adrenaline have been shown to be associated with endothelial dysfunction, coagulopathy and increased mortality (139) (140). A retrospective study noted a greater incidence of atrial fibrillation

associated with catecholamine use (141). Although, Arababi et al also demonstrated an improvement in mortality, the results from the retrospective study by Nieeden et al 2008 demonstrated contradictory results (96,98,99).

4.2 Study Aims

Although, the deleterious effects of catecholamines have been alluded to in polytrauma, their association with adverse cardiac events has only been explored retrospectively. Study data collected during TACID demonstrated the elevation of cardiac enzymes following traumatic injury. Release of these enzymes is suggestive of cardiomyocyte death in this cohort. The aim of this chapter is to explore the contribution of catecholamines to cardiac injury as well as length of stay and mortality in this patient group. Plasma levels of adrenaline, noradrenaline and dopamine concentrations were measured on admission, at 24 hours and 72 hours following injury.

4.3 Results

4.3.1 Plasma Catecholamine in Adverse Cardiac Events

On admission plasma adrenaline and noradrenaline levels were significantly higher in the group who developed ACE. Although, the distribution of the admission plasma dopamine levels is skewed, this was not significantly different. The ACE group had a median noradrenaline concentration of 2.03µg/ml, twice that of the no ACE group at 1.08 µg/ml (normal reference 0.19–0.23 ng/mL). The adrenaline

concentration on admission was three times higher at 0.67 μg/ml compared to the no ACE group at 0.26 μg/ml (normal reference 0.06–0.08 ng/mL). Although the dopamine concentration on admission in the ACE group was higher 0.0031 μg/ml as compared to 0.0018 μg/ml, this difference was not significantly different (normal reference 0.05–0.1 ng/mL).

At 24 hours following injury, there was a significant difference in the concentration of noradrenaline and dopamine but not adrenaline. The difference in plasma noradrenaline concentration became much more apparent with a concentration of 5.11 μg/ml in the ACE group versus 0.88 μg/ml in the no ACE group - representing a six fold increase. The plasma adrenaline concentration at 24 hours is similar between the two groups with 0.095 μg/ml in ACE and 0.107 in the no ACE group. Dopamine concentration only demonstrates a difference at the 24-hour time point. The concentration of dopamine is three times greater in the ACE group ay 0.0269 μg/ml compared to 0.0083 μg/ml in the no ACE group.

At 72 hours, the only demonstrable difference in circulating catecholamines was the plasma adrenaline concentration. The ACE group had four times greater at 3.22 μg/ml compared to 0.814 μg/ml. This may be due to the exogenous catecholamine use.

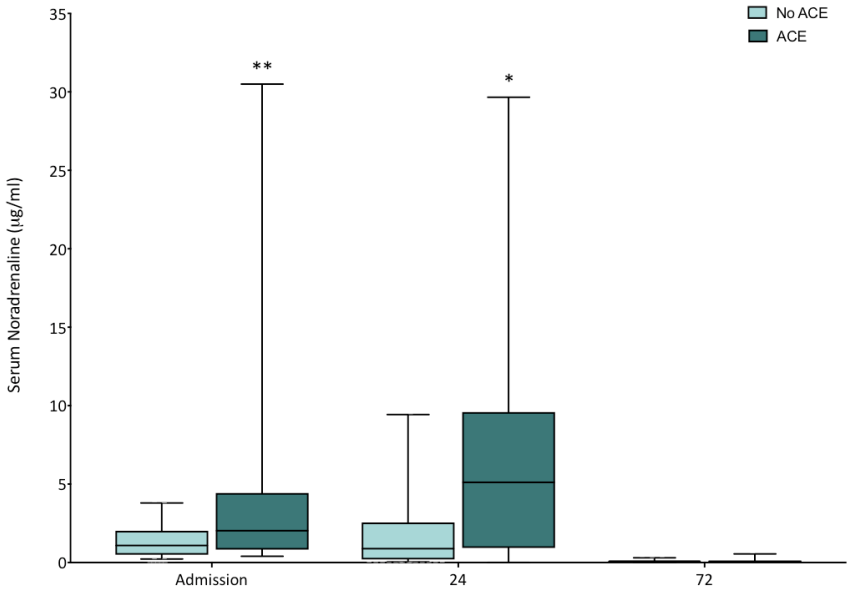


Figure 4.1 *Boxplot showing difference in noradrenaline (NA) levels with the presence of ACE- On admission the median serum levels were 1.08 µg/ml (IQR: 0.49-2.0) in the no ACE group vs. median 2.03 µg/ml (IQR: 0.82- 4.3) in ACE (p<0.01). At 24h- 0.89 µg/ml (IQR: 0.20- 2.52) in the no ACE group vs. 5.11µg/ml (IQR: 0.93-9.59) in ACE (p<0.01). At 72h- 0.07 µg/ml (IQR: 0.04-0.13) in the no ACE group vs 0.07 µg/ml (IQR: 0-0.13) in ACE (p=0.68).*

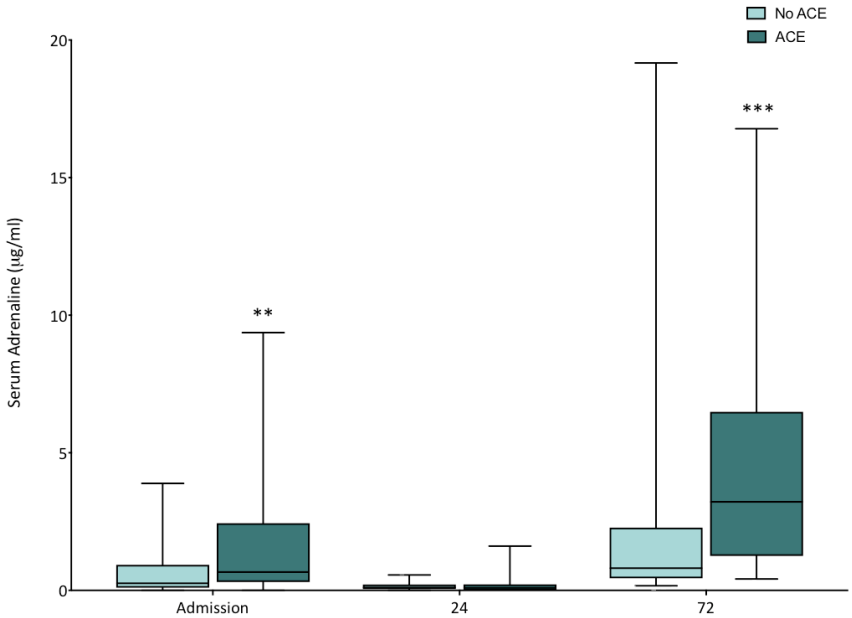


Figure 4.2 *Boxplot showing difference in adrenaline levels with the presence of ACE- On admission the median serum levels were 0.26 µg/ml (IQR: 0.49-2.0) in the no ACE group vs. 0.67 µg/ml (IQR: 0.30- 2.44) in ACE (p=0.01). At 24h- 0.11 µg/ml (IQR: 0.46-0.21) in the no ACE group vs. 0.10 (IQR: 0-0.22) in ACE (p=0.6). At 72h- 0.81 µg/ml (IQR: 0.44-2.28) in the no ACE group vs 3.22 µg/ml (IQR: 1.25-6.49) in ACE (p<0.01).*

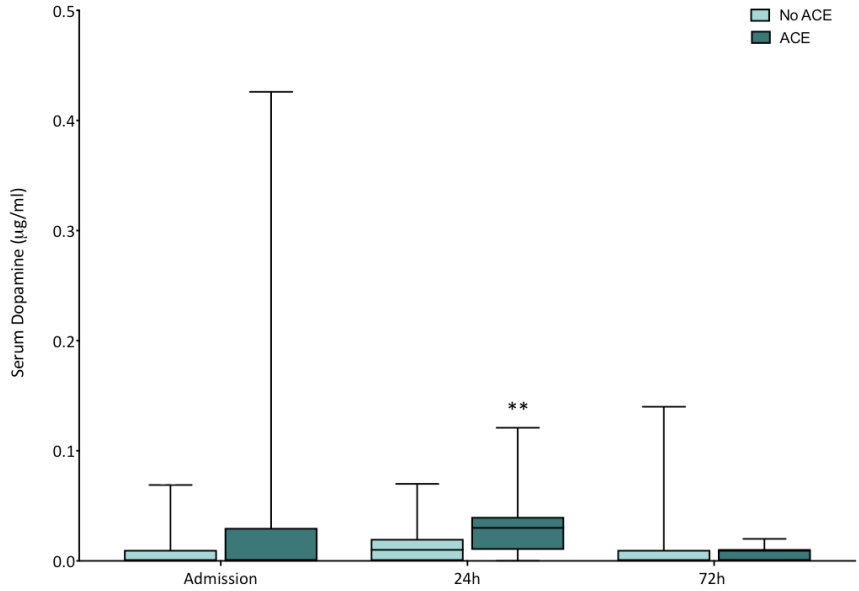


Figure 4.3 *Boxplot showing difference in dopamine levels with the presence of ACE- On admission the median serum levels were 0.00.18 µg/ml (IQR: 0-0.008) in the no ACE group vs. 0.0031 µg/ml (IQR: 0- 0.025) in ACE (p=0.09). At 24h- 0.008 µg/ml (IQR: 0.003-0.018) in the no ACE group vs. 0.027 (IQR: 0-.013-0.412) in ACE (p<0.01). At 72h- 0.004 µg/ml (IQR: 0-0.010) in the no ACE group vs 0.006 µg/ml (IQR: 0-0.009) in ACE (p<0.01).*

In summary on admission, plasma noradrenaline and adrenaline were significantly higher in the ACE group at baseline, with noradrenaline remaining higher at 24 hours. Adrenaline remains elevated in the ACE group at 72 hours, potentially due to exogenous administration of inotropes.

4.3.2 Catecholamines and Injury Burden

We then investigated the association between circulating levels of catecholamines and the severity of injury patients had sustained. An ISS of greater than 15 was used to identify severely injured patients. The severely injured patients had higher plasma noradrenaline levels on admission compared to the less injured group (1.2 µg/ml vs. 0.99µg/ml, p=0.01). This difference was sustained and becomes more marked at 24 hours; with plasma levels of 1.7 µg/ml in the more injured group almost double that on the less injured group at 0.9µg/ml (p=0.01). However, at 72 hours, the levels of noradrenaline had diminished in both groups and no evident difference was detectable between the two groups (Figure 4.2).

Plasma adrenaline was also raised on admission and the severely injured group displayed more than double the circulating levels demonstrated in the less injured cohort (0.5 µg/ml vs. 0.2µg/ml respectively (p=0.001)). Serum adrenaline levels reduced at 24 hours in both groups and then increased at 72hrs with median levels in the severely injured patients more than double that of the less injured patients (1.4 µg/ ml vs. 0.6 µg/ml (p=0.001)). The change at 72 hours is secondary to exogenous adrenaline administration rather that a physiological response.

Dopamine levels are also raised on admission and higher in the more severely injured group 0.003 µg/ml, whereas the median in the less injured group in 0 µg/ml (p=0.008). The dopamine levels rise further at 24 hours, with levels in the severely

injured group, double that of the less injured group, 0.014 μg/ml vs 0.007 μg/ml ($p<0.001$).

We then dichotomised patients according to the head AIS (≤3 vs. >3) in order to evaluate whether sever head injury had a contributory effect on the level of circulating catecholamines (Figure 4.3). When the two groups were compared there was no difference seen in any of the plasma catecholamines levels.

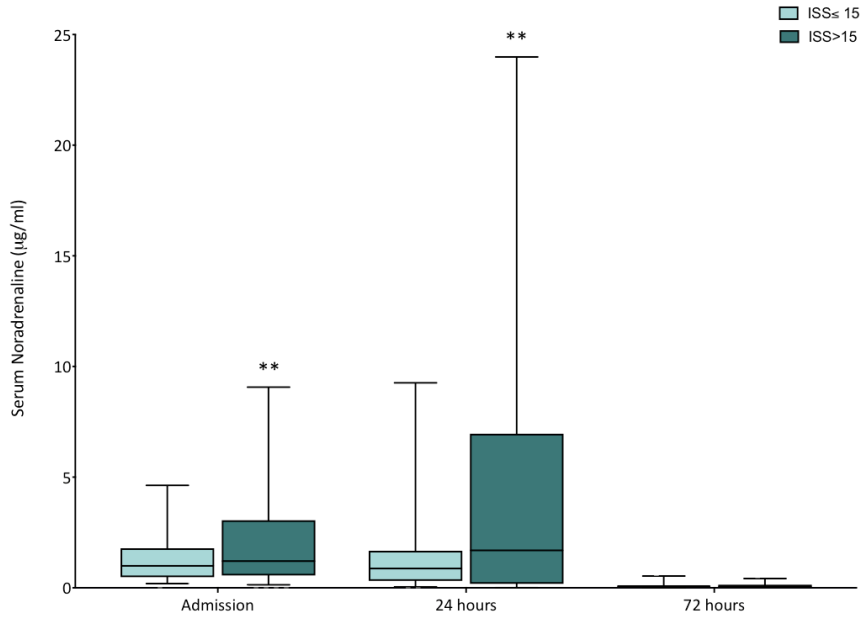


Figure 4.4 *Boxplot showing difference in noradrenaline levels in severely injured patients as defined by ISS>15- On admission the median serum levels were 0.99 µg/ml (IQR: 0.48-1.79) in the ISS≤15 group vs. 1.21 µg/ml (IQR: 0.56- 3.05) in ISS>15 (p=0.01). At 24h- 0.87 µg/ml (IQR: 0.31-1.68) in the ISS≤15 group vs. 1.69 (IQR: 0.18-6.96) in ISS>15 (p=0.01). At 72h- 0.07 µg/ml (IQR: 0.05-0.11) in the ISS≤15 group vs 0.06 µg/ml (IQR: 0-0.14) in ISS>15 (p=0.9).*

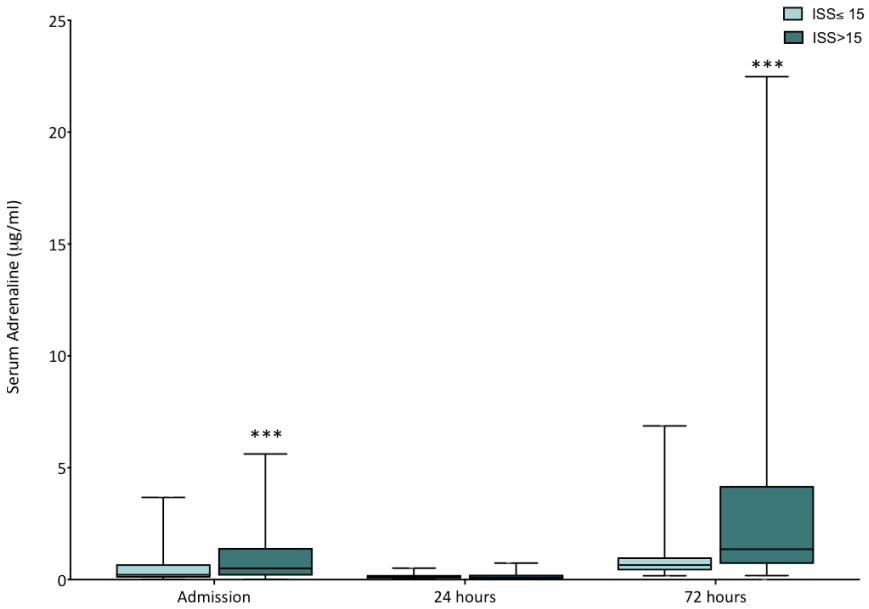


Figure 4.5 *Boxplot showing difference in adrenaline levels in severely injured patients as defined by ISS>15- On admission the median serum levels were 0.21 µg/ml (IQR: 0.09-0.69) in the ISS≤15 group vs. 0.50 µg/ml (IQR: 0.19- 1.41) in ISS>15 (p=0.01). At 24h- 0.11 µg/ml (IQR: 0.05-0.21) in the ISS≤15 group vs. 0.10 (IQR: 0.03-0.22) in ISS>15 (p=0.01). At 72h- 0.07 µg/ml (IQR: 0.003-0.014) in the ISS≤15 group vs 1.36 µg/ml (IQR: 0.70-4.19) in the ISS>15 group (p<0.01)*

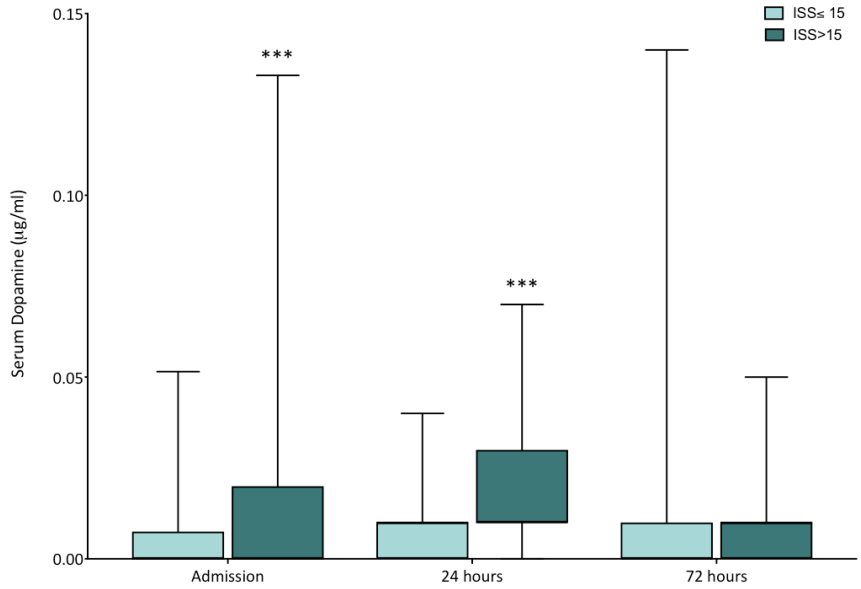


Figure 4.6 *Boxplot showing difference in dopamine levels in severely injured patients as defined by ISS>15- On admission the median serum levels were 0 µg/ml (IQR: 0-0.006) in the ISS≤15 group vs. 0.003 µg/ml (IQR: 0- 0.018) in the ISS>15 (p<0.01). At 24h- 0.007 µg/ml (IQR: 0.003-0.014) in the ISS≤15 group vs. 0.015 (IQR: 0.005-0.031) in ISS>15 (p<0.01). At 72h- was 0.003 µg/ml (IQR: 0-0.008) in the ISS≤15 group vs 0.006 µg/ml (IQR: 0-0.12) in ISS>15 (p=0.2).*

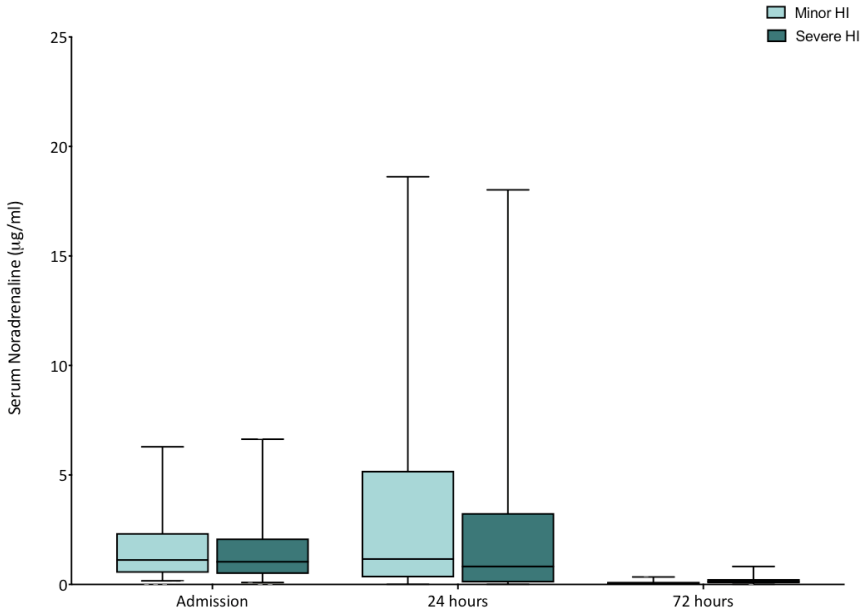


Figure 4.7 *Boxplot showing difference in noradrenaline levels in severe head injury as defined by head and neck AIS>3 - On admission the median serum levels were 1.12 µg/ml (IQR: 0.53-2.35) in minor HI vs. median 1.04 µg/ml (IQR: 0.48- 2.11) in severe HI (p=0.8). At 24h- 1.16 µg/ml (IQR: 0.32-5.19) in minor HI vs. 0.82 (IQR: 0.09-3.26) in severe HI (p=0.2). At 72h- 0.06 µg/ml (IQR: 0-0.12) in minor HI vs 0.13 µg/ml (IQR: 0.06-0.25) in severe HI (p=0.1).*

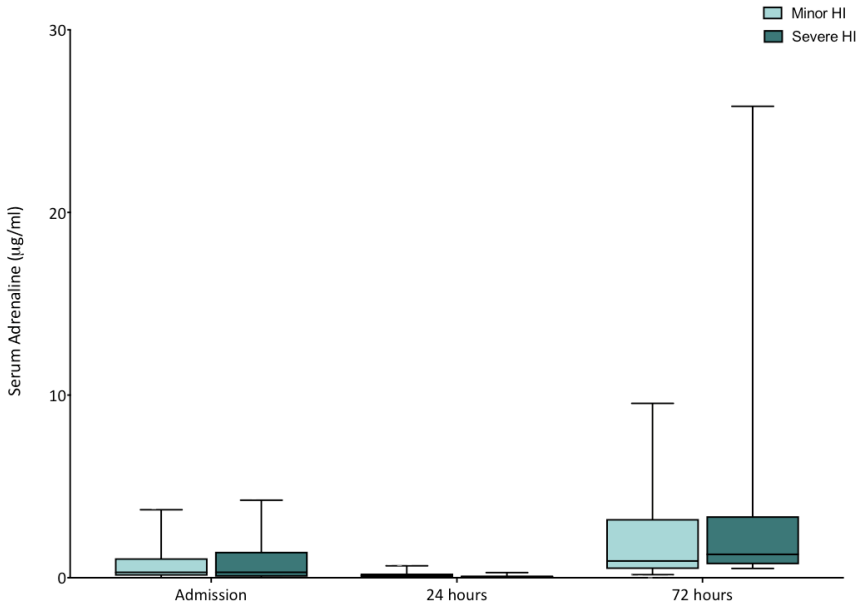


Figure 4.8 *Boxplot showing difference in adrenaline levels in severe head injury as defined by head and neck AIS>3 - On admission the median serum levels were 0.3 µg/ml (IQR: 0.11-1.07) in minor HI vs. median 0.30 µg/ml (IQR: 0.06- 1.43) in severe HI (p=0.8). At 24h- 0.11 µg/ml (IQR: 0.05-0.23) in minor HI vs. 0.07 (IQR: 0.01-0.11) in severe HI (p=0.06). At 72h- 0.92 µg/ml (IQR: 0.48-3.22) in minor HI vs 1.28 µg/ml (IQR: 0.74-3.37) in severe HI (p=0.2).*

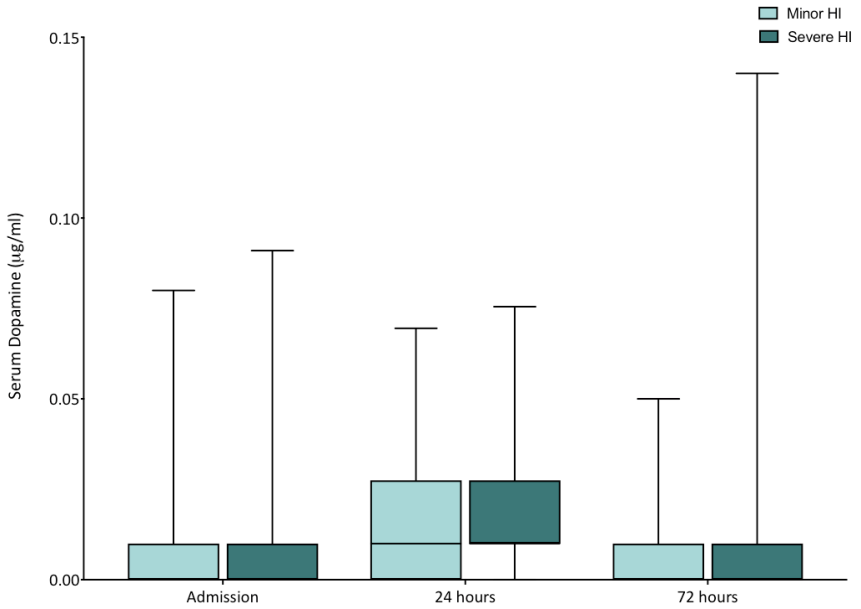


Figure 4.9 *Boxplot showing difference in dopamine levels in severe head injury as defined by head and neck AIS>3 - On admission the median serum levels were 0.003 µg/ml (IQR: 0-0.012) in minor HI vs. 0 µg/ml (IQR: 0- 0.007) in severe HI (p=0.2). At 24h- 0.010 µg/ml (IQR: 0.004-0.03) in minor HI vs. 0.014 (IQR: 0.006-0.024) in severe HI (p=0.6). At 72h- 0.005 µg/ml (IQR: 0-0.01) in minor HI vs 0.003 µg/ml (IQR: 0-0.014) in severe HI (p=0.7).*

4.3.4 Catecholamine levels and cardiac injury

To further investigate the relationship between catecholamine levels and the development of cardiac injury, plasma catecholamine levels were divided into quartiles and compared to circulating h-FABP as a previously defined marker of cardiac injury. The change in admission catecholamine level was compared to that of the levels of plasma h-FABP on admission, at 24 hours and 72 hours. Increasing levels of catecholamine exposure on admission is associated with increasing levels of h-FABP in the plasma. On admission, increasing adrenaline concentration occurs in line with a concurrent increase in h-FABP concentration. However, on admission and at 24 hours, only the difference between the 1st and 4th quartile as well as the difference between the 2nd and 4th quartile of adrenaline concentration is statistically significant. At 72 hours, only the difference between the 1st and 4th quartile is statistically different.

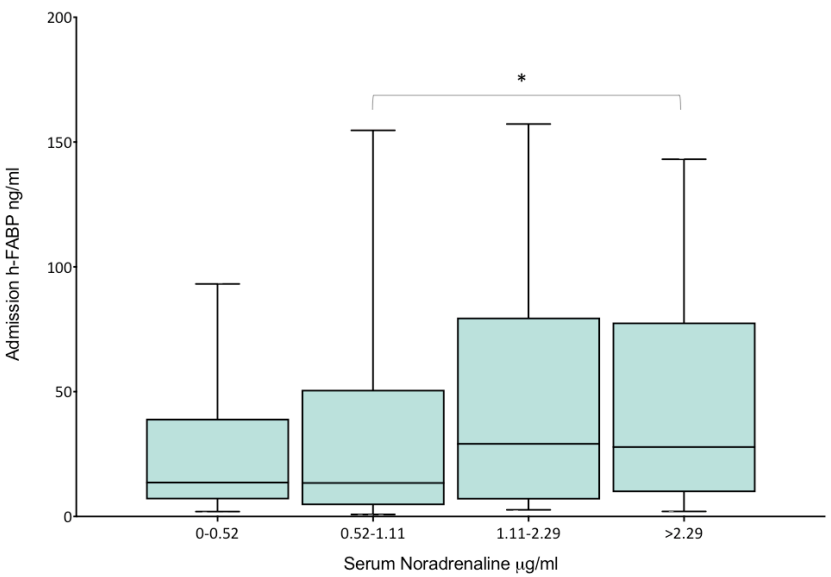
With noradrenaline and dopamine the 1st quartile median is higher than the second quartile but from there on there is a stepwise increase in h-FABP concentration. At all time points, the concentration of h-FABP is statistically significant between the 2nd and 4th quartile. The only statistical difference in h-FABP levels in comparison with Dopamine concentration, is at 24 hours between the 1st and 4th Quartile.

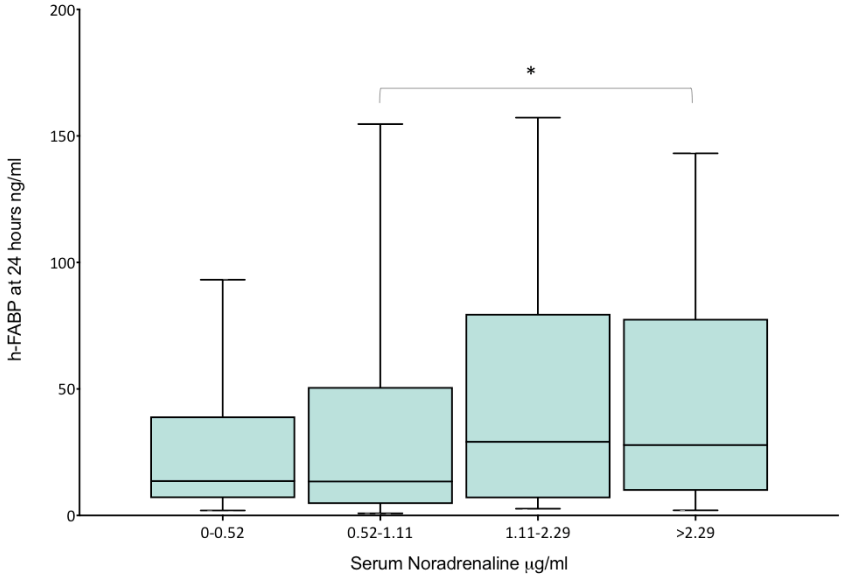
Table 4.1: Summary of median serum hFABP (ng/ml) within the different quartiles of catecholamines

	Noradrenaline Quartiles			
	0-0.52	**0.52-1.11**	**1.11-2.29**	**>2.29**
Admission	13.64	13.44	29.14	27.87
24 hours	9.27	4.95	16.42	32.19
72 hours	2.37	2.31	7.18	38.29

	Adrenaline Quartiles			
	0-0.11	**0-0.11-0.030**	**0.30-1.09**	**>1.09**
Admission	9.13	13.04	22.49	43.23
24 hours	9.27	4.95	16.42	32.19
72 hours	2.04	5.215	5.135	8.835

	Dopamine Quartiles			
	0-0.0001	**0.0001-0.002**	**0.002-0.0107**	**>0.0107**
Admission	17.03	10.59	18.62	59.37
24 hours	9.515	4.27	17.98	131.4
72 hours	2.37	2.31	7.175	38.29





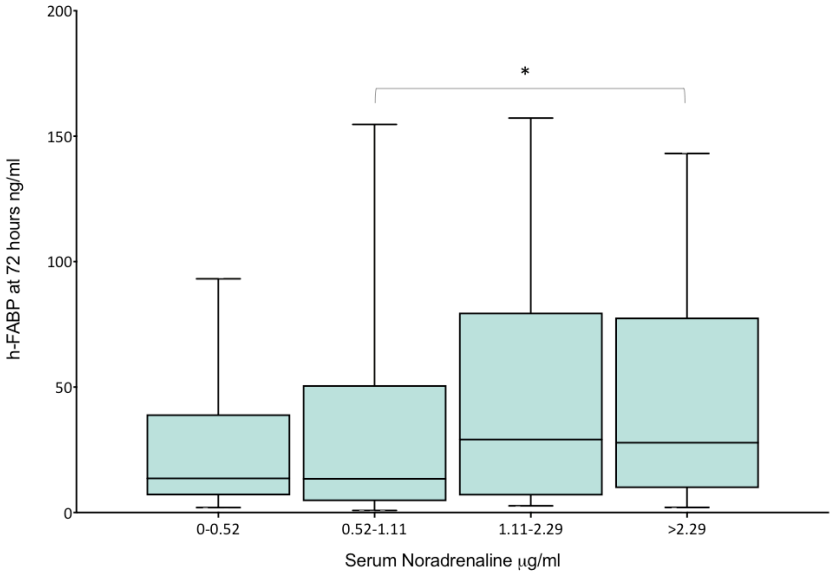
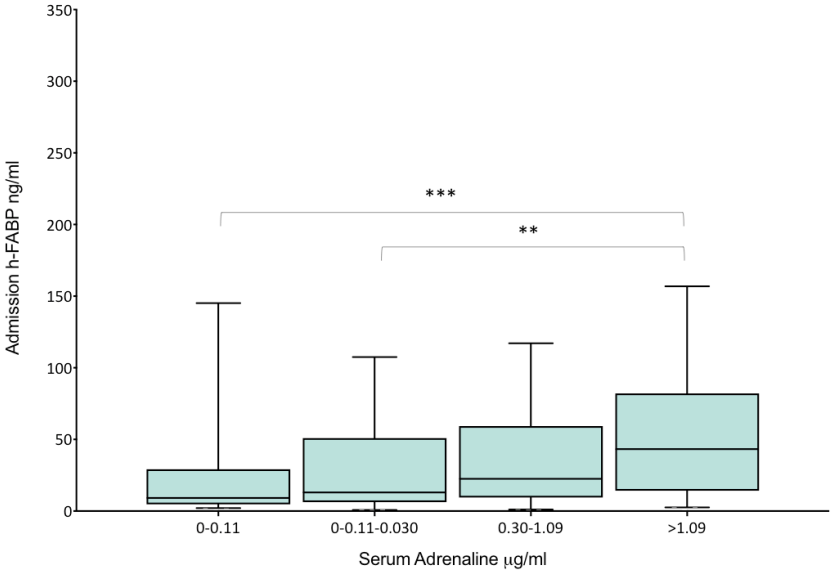
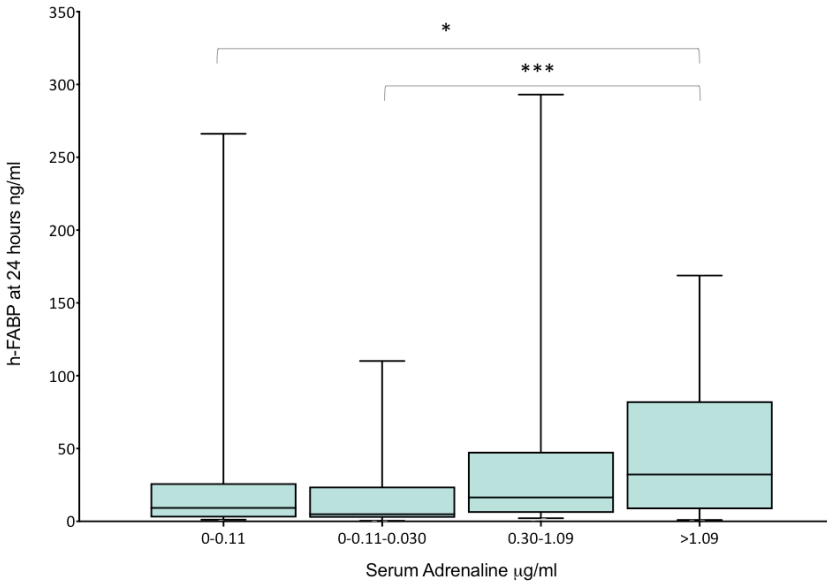


Figure 4.10 *Boxplot graph showing changes in h-FABP levels with increasing levels of noradrenaline. On admission (p=0.04*), at 24 hours (p=0.04) and at 72 hours (p=0.04) there is a significant difference between the 2nd and 4th quartile.*





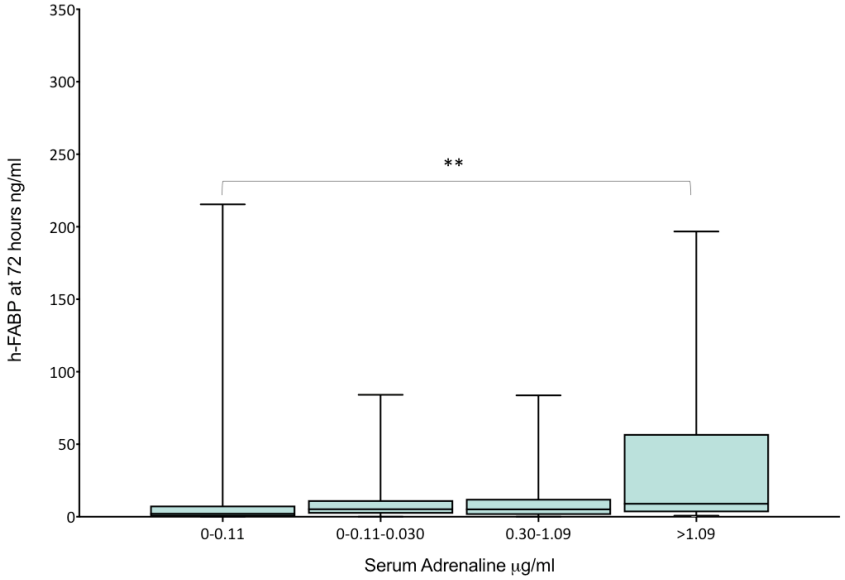
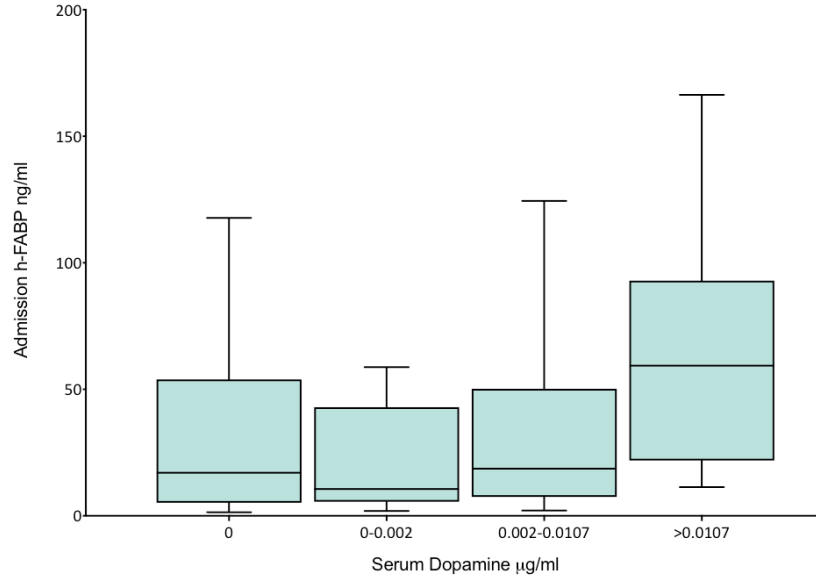
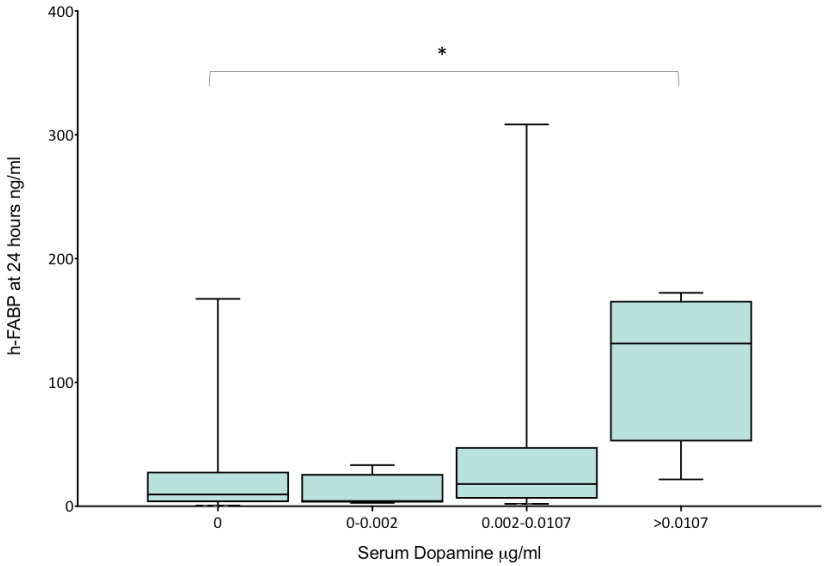


Figure 4.11 *Boxplot graph showing changes in h-FABP levels with increasing levels of adrenaline. On admission (p<0.001***), at 24 hours (p=0.04*) and at 72 hours (p=0.002**) there is a significant difference between the 1st and 4th quartile. On admission (p=0.008**) and at 24 hours (p<0.001***) there is also a significant difference between 2nd and 4th quartile.*





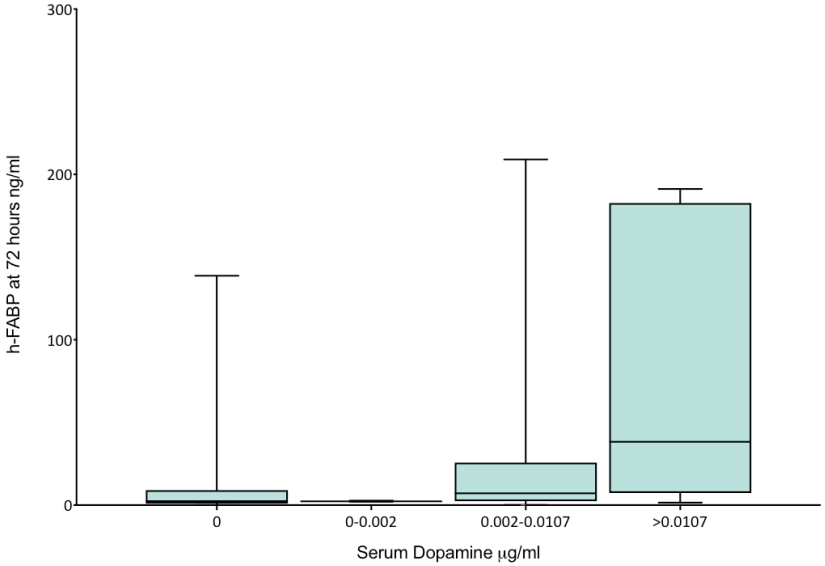


Figure 4.11 *Boxplot graph showing changes in h-FABP levels with increasing levels of dopamine. There is a significant difference between the 1st and 4th quartile (p=0.04*). On admission and at 24 hours there is also a significant difference between 2nd and 4th quartile.*

Further analysis was conducted by comparison of the plasma levels of catecholamines to the presence of TISCI, as defined by plasma h-FABP of greater than 16.8 ng/ml, at the corresponding time points. On admission, the median plasma noradrenaline level of 1.4 μg/ml was 1.5 times higher than in the no TISCI group at 0.9 μg/ml, (p=0.001). The admission adrenaline level was three times higher at 0.6 μg/ml compared to 0.2 μg/ml in the group without TISCI ($p < 0.001$).

Although there is a difference in plasma noradrenaline levels at 24 hours, with the median in the TISCI group of 1.5 μg/ml and the median in no TISCI group of 0.9 μg/ml, this difference is not statistically significantly. The dopamine level at 24 hours is a third higher at 0.012 μg/ml compared to 0.009 μg/ml in the no TISCI group (p= 0.03). At 72 hours, adrenaline levels are significantly higher at 1.4 μg/ml vs. 0.9 μg/ml (p= 0.03).

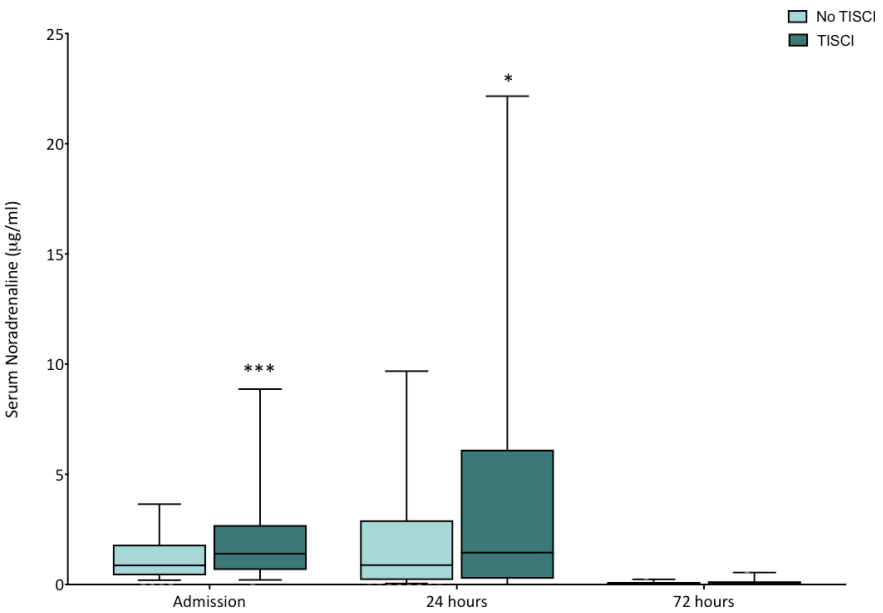


Figure 4.13 *Boxplot showing difference in noradrenaline levels with TISCI - On admission the median serum levels were 0.87 µg/ml (IQR: 0.42-1.81) without TISCI vs. 1.40 µg/ml (IQR: 0.66- 2.70) with TISCI (p=0.001***). At 24h median was 0.82 µg/ml (IQR: 0.18-2.16) without TISCI vs. 1.50 (0.38-6.00) with TISCI (p=0.03*). At 72h the median was 0.06 µg/ml (0.04-0.11) without TISCI vs 0.08 µg/ml (IQR:0.04-0.14) with TISCI (p=0.4).*

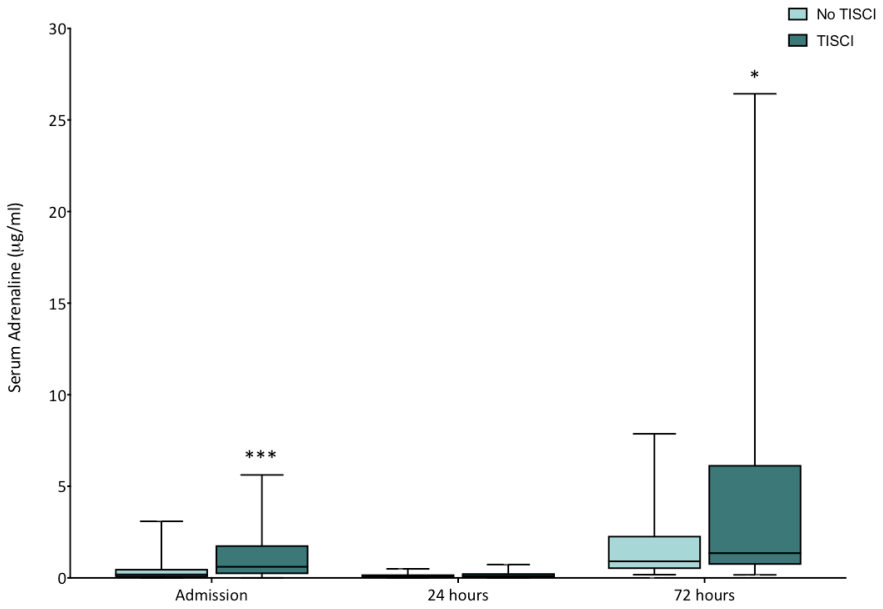


Figure 4.14 *Boxplot showing difference in adrenaline levels with TISCI - On admission the median serum levels were 0.19 µg/ml (IQR: 0.05-0.5) without TISCI vs. 0.61 µg/ml (IQR: 0.20- 1.79) with TISCI (p<0.001***). At 24h median was 0.10 µg/ml (IQR: 0.04-0.21) without TISCI vs. 0.11 (IQR: 0.05-0.22) with TISCI (p=0.9). At 72h the median was 0.74 µg/ml (IQR: 0.35-1.25) without TISCI vs 1.35 µg/ml (IQR:0.65- 4.31) with TISCI (p=0.003**).*

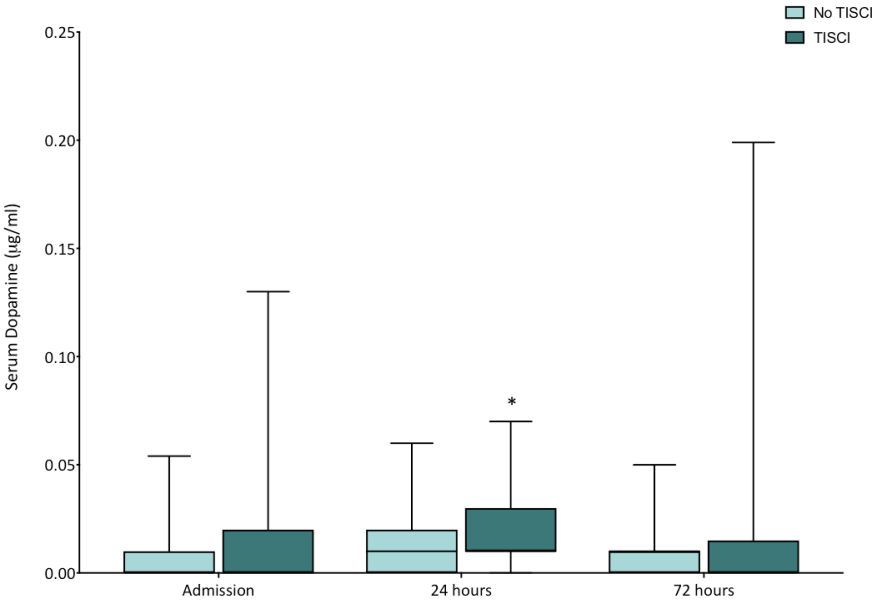


Figure 4.15 *Boxplot showing difference in dopamine levels in TISCI - On admission the median serum levels were 0.001µg/ml (IQR: 0-0.008) without TISCI group vs. median 0.003 µg/ml (IQR: 0- 0.018) in TISCI group (p=0.2). At 24h median was 0.01 µg/ml (IQR: 0.004-0.016) without TISCI vs. 0.01 (IQR: 0.005-0.029) with TISCI (p=0.1). At 72h the median was 0.003 µg/ml (IQR: 0-0.008) without TISCI vs 0.005 µg/ml (IQR:0- 0.012) with TISCI (p=0.2).*

4.3.5 Catecholamines and Shock

Severe physiological stress leads to the initiation of the sympathetic system and leads to a hyperadrenergic state. This was demonstrated in this trauma population. A plasma lactate level of greater than 2mmol/l was taken as a surrogate for hypoperfusion and therefore shock. The shocked group had higher levels of plasma noradrenaline on admission (1.5 µg/ml vs. 0.9 µg/ml, p< 0.001). Plasma adrenaline was higher at 72 hours (1.4 µg/ml vs 0.8 µg/ml, p=0.002). Admission dopamine levels were higher in the shocked group (0.013 µg/ml vs. 0.008 µg/ml, p= 0.04).

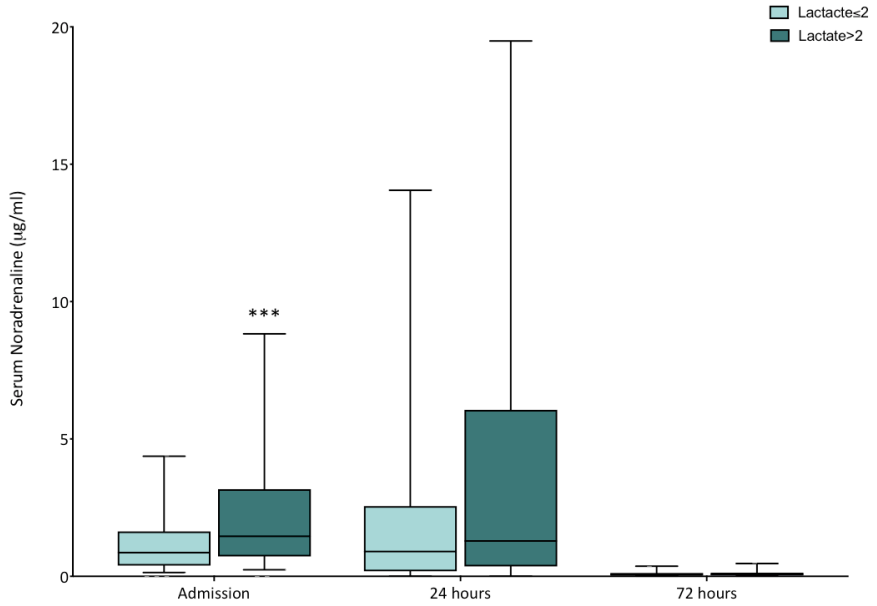


Figure 4.16 *Boxplot showing difference in noradrenaline levels using lactate as a marker of shock - On admission the median serum levels were 0.87µg/ml (IQR: 0.39- 0.1.64) in the not shocked group vs. 1.46µg/ml (IQR: 0.73- 3.18) in shocked (p<0.001***). At 24h- 0.91µg/ml (IQR: 0.18-2.56) in the not shocked group vs. 1.29 (IQR: 0.36- 6.06) in shocked (p=0.1). At 72h- 0.06µg/ml (IQR: 0.04-0.13) in the not shocked group vs 0.07 µg/ml (IQR:0.04- 0.14) in shocked (p=0.7).*

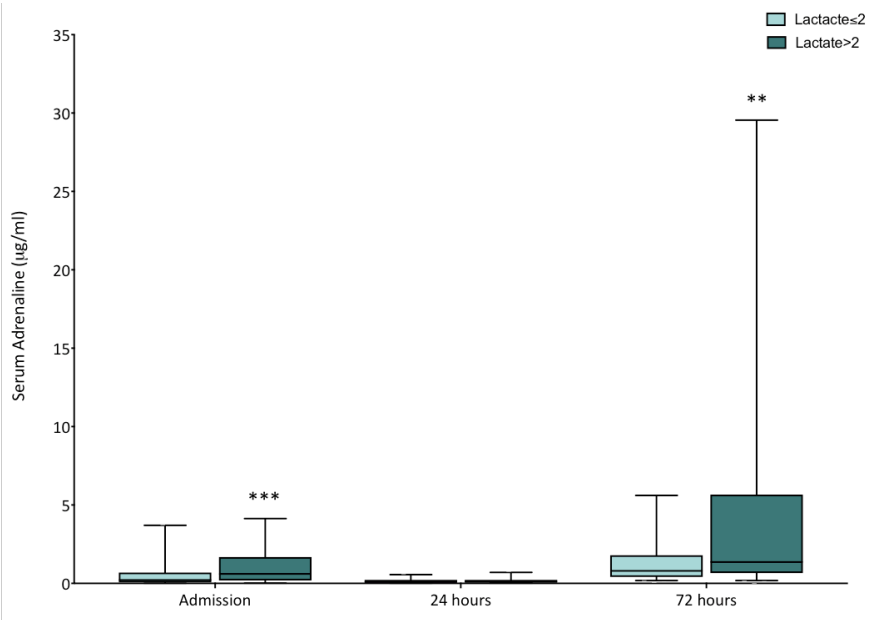


Figure 4.17 *Boxplot showing difference in adrenaline levels using lactate as a marker of shock - On admission the median serum levels were 0.22µg/ml (IQR: 0.06- 0.69) in the not shocked group vs. 0.60µg/ml (IQR: 0.18- 1.67) in the shocked (p<0.001***). At 24h- 0.09µg/ml (IQR: 0.05-0.22) in the not shocked group vs. 0.11 (IQR: 0.04- 0.21) in shocked (p=0.4). At 72h-0.80µg/ml (IQR: 0.41-1.80) in the not shocked group vs 1.35 µg/ml (IQR: 0.65- 5.65) in shocked (p=0.002**).*

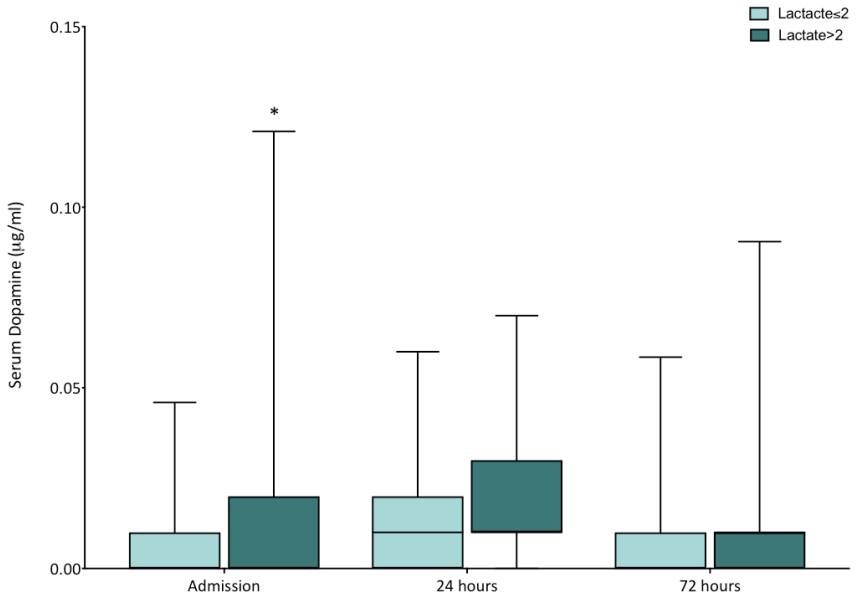


Figure 4.18 *Boxplot showing difference in dopamine levels using lactate as a marker of shock - On admission the median serum dopamine levels were 0µg/ml (IQR: 0- 0.007) in the not shocked group vs. 0.003µg/ml (IQR: 0- 0.02) in the shocked group (p=0.04*). At 24h- 0.008µg/ml (IQR: 0.004-0.21) in the not shocked group vs. 0.013 (IQR: 0.006- 0.03) in shocked (p=0.08). At 72h- 0.004µg/ml (IQR: 0-0.009) in the not shocked group vs 0.005µg/ml (IQR: 0- 0.01) in shocked (p=0.9).*

4.3.6 Inotrope administration and Catecholamine levels

The difference in plasma catecholamine levels at 24 hours and 72 hours could have been attributable to the administration of exogenous inotropes. In the patients who developed ACE, 76% received inotropic support in comparison to 15% in the cohort did not develop ACE and 23% as a whole population. Of the patients who received inotropes, 81% received noradrenaline alone, where as 3% received adrenaline, 9% dobutamine, 7% Dopexamine and 4% milrinone. In the no ACE group, the only inotropes given was noradrenaline and dopexamine. In this group, 36 (12%) patients received inotropes and of those 92 % received noradrenaline only. The reasons for catecholamine administration were not collected. However, 49% of the patients who received noradrenaline had significant head injuries and the other 51% were severely injured with a median ISS of 34.

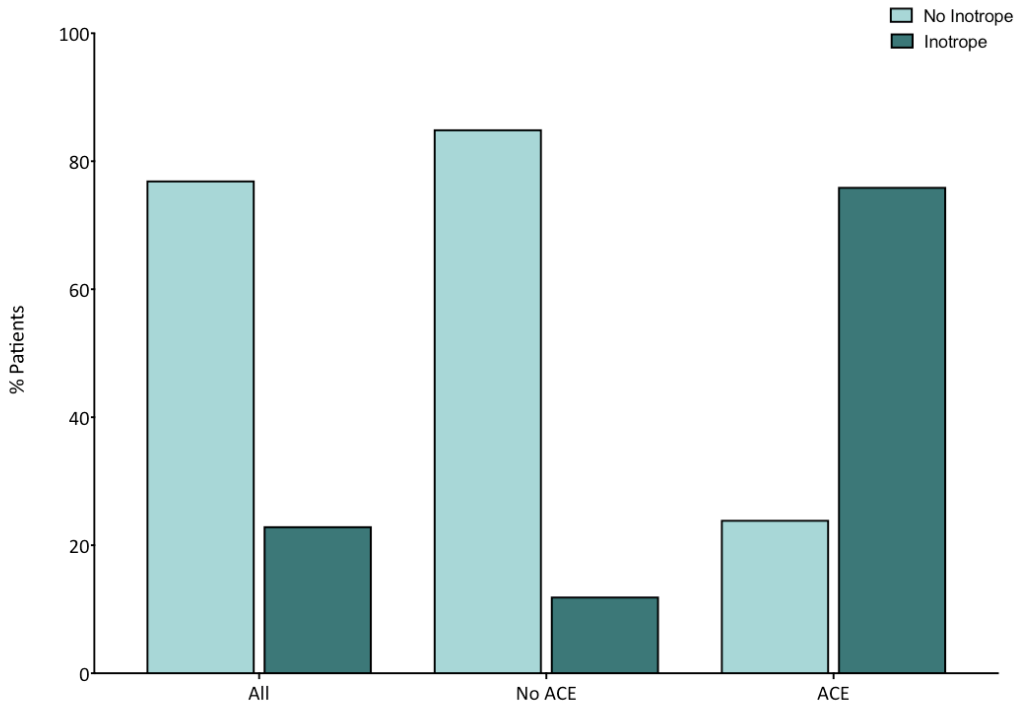


Figure 4.6: *Comparison of inotrope requirement in the ACE and no ACE group.*

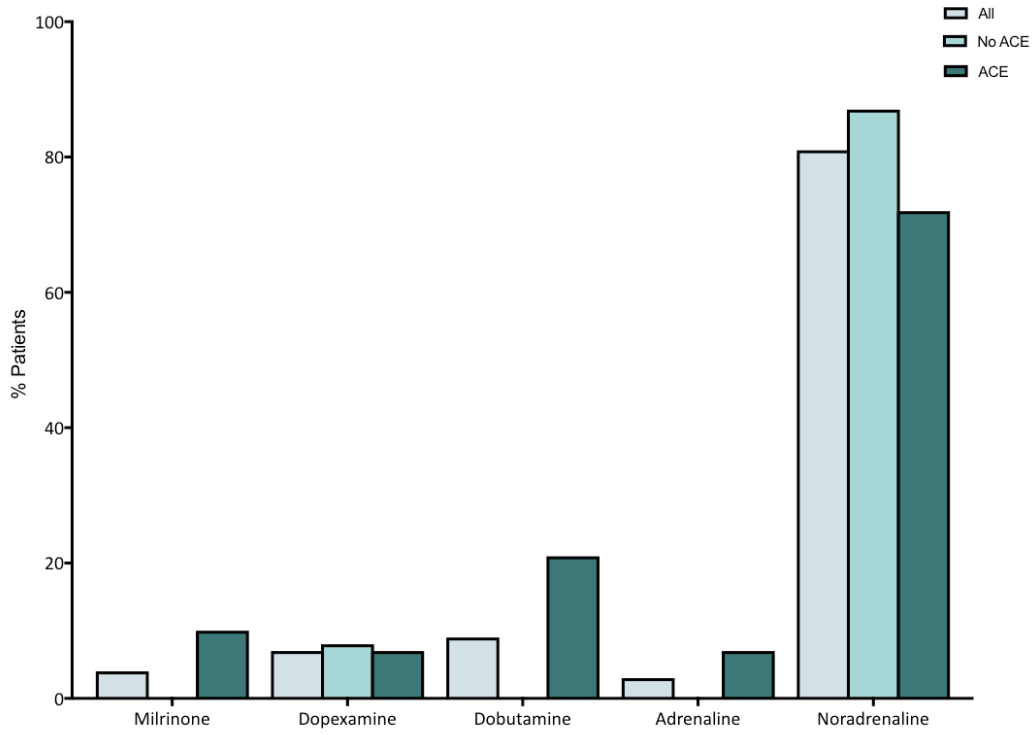


Figure 4.7: *Comparison of inotropes initiated in the ACE and no ACE group.*

None of the patients included in this study received inotropes prior to baseline venepuncture; therefore the admission plasma catecholamines levels can be attributed to endogenous catecholamines production. The patients who require inotropic support during their stay have higher admission levels of noradrenaline, adrenaline and dopamine (Figure 4.8). The high admission catecholamine levels may be a reflection of greater injury severity that consequentially leads organ dysfuction requiring inotropic support or it may be that the high levels of catecholamine exposure lead to cardiac injury and dysfunction requiring inotropic support.

The serum catecholamine levels at 24 and 72 hours demonstrates exogenous and endogenous catecholamines. Catecholamine use is well established in the management of organ injury in critically injured patients (142). Noradrenaline and Dopamine levels are higher at 24 hours in the group of patients receiving inotropes and Adrenaline levels higher at 72 hours in patients receiving inotropes. It is difficult to ascertain from this data whether the development of ACE is the cause or effect of inotrope use.

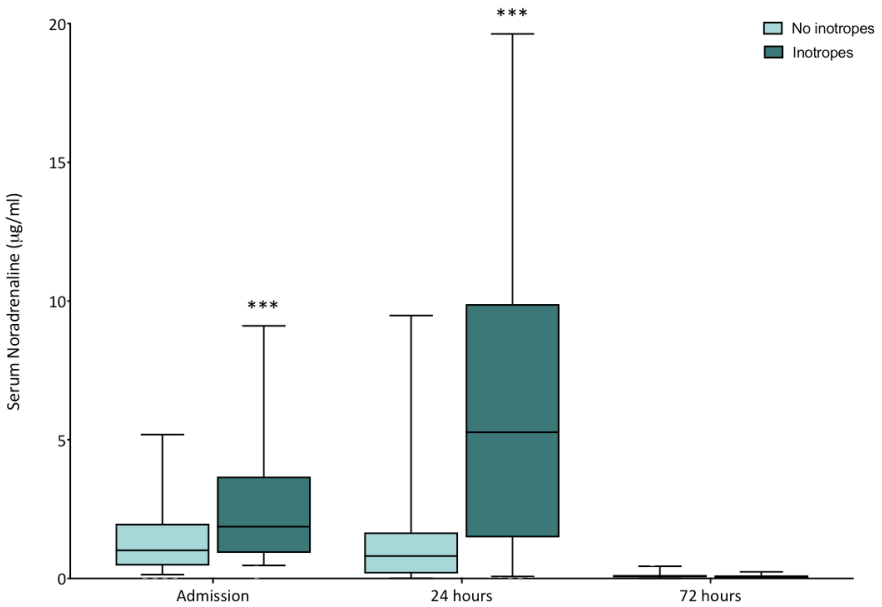


Figure 4.19 *Boxplot showing difference in noradrenaline levels with inotrope administration - On admission the median serum levels were 1.01µg/ml (IQR: 0.47- 1.97) without inotrope use vs.1.87µg/ml (IQR: 0.92- 3.68) with inotropes (p<0.001***). At 24h- 0.81µg/ml (IQR: 0.18-1.66) without inotropes vs. 5.27 (IQR: 1.48- 9.89) with inotropes(p<0.001***). At 72h- 0.08µg/ml (IQR: 0.04-0.13) without inotropes vs 0.06 µg/ml (IQR: 0- 0.12) with inotropes (p=0.2).*

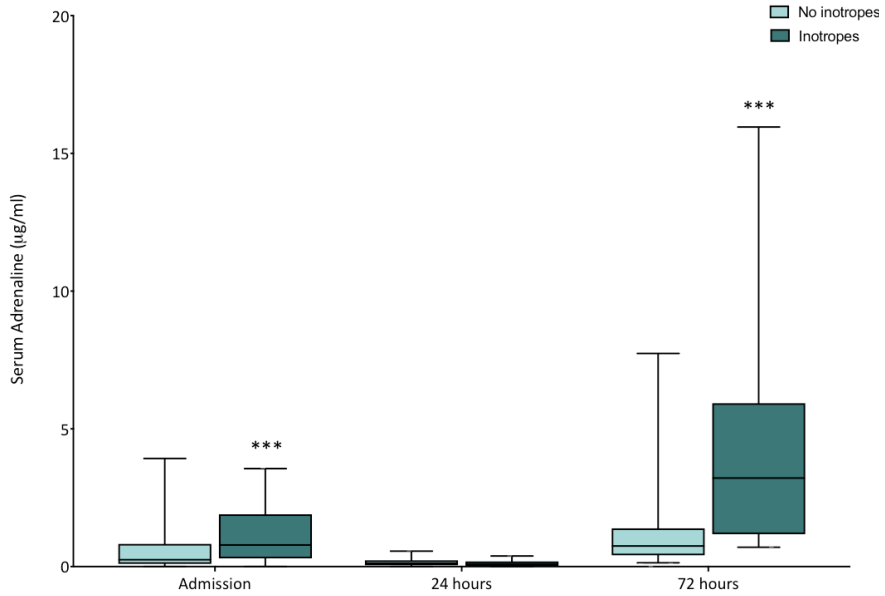


Figure 4.20 *Boxplot showing difference in adrenaline levels with inotrope administration - On admission the median serum levels were 0.25µg/ml (IQR: 0.10- 0.83) without inotrope use vs. 0.78µg/ml (IQR: 0.30- 1.90) with inotropes (p<0.001***). At 24h- 0.12µg/ml (IQR: 0.05-0.23) without inotropes vs. 0.09 (IQR: 0.009- 0.19) with inotropes (p=0.25). At 72h- 0.75µg/ml (IQR: 0.41-1.39) without inotropes vs 3.22 µg/ml (IQR: 1.18- 5.93) with inotropes (p<0.001***).*

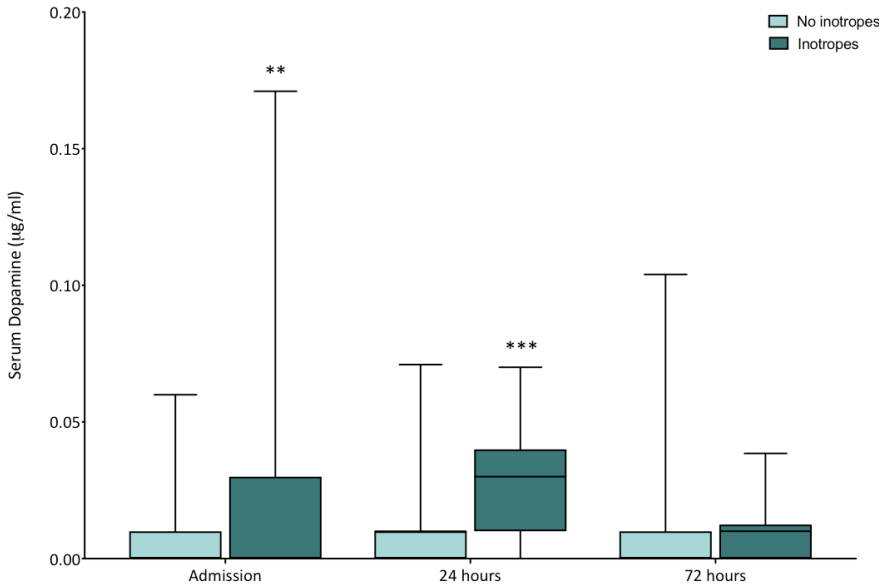


Figure 4.21 *Boxplot showing difference in noradrenaline levels with inotrope administration - On admission the median serum levels were 0µg/ml (IQR: 0-0.007) withour inotrope use vs. median 0.004µg/ml (IQR: 0- 0.03) with inotropes (p=0.005**). At 24h- 0.007µg/ml (IQR: 0.004-0.015) without inotropes vs. 0.03 (IQR: 0.01- 0.04) with inotropes (p<0.001***). At 72h- 0.004µg/ml (IQR: 0- 0.009) without inotrope use vs 0.007 µg/ml (IQR: 0- 0.02) with inotrope (p=0.2).*

4.3.7 Catecholamine levels and mortality

The difference in catecholamine levels were examined to determine whether its deleterious effects extended to mortality.

Admission noradrenaline levels in non-survivors were almost twice that seen in survivors, 1.74 µg/ml vs 1.09 µg/ml. This difference became significantly greater at 24 hours with noradrenaline levels being nine times greater at 8.08 µg/ml compared to 0.91 µg/ml in survivors. However at 72 hours this difference diminishes.

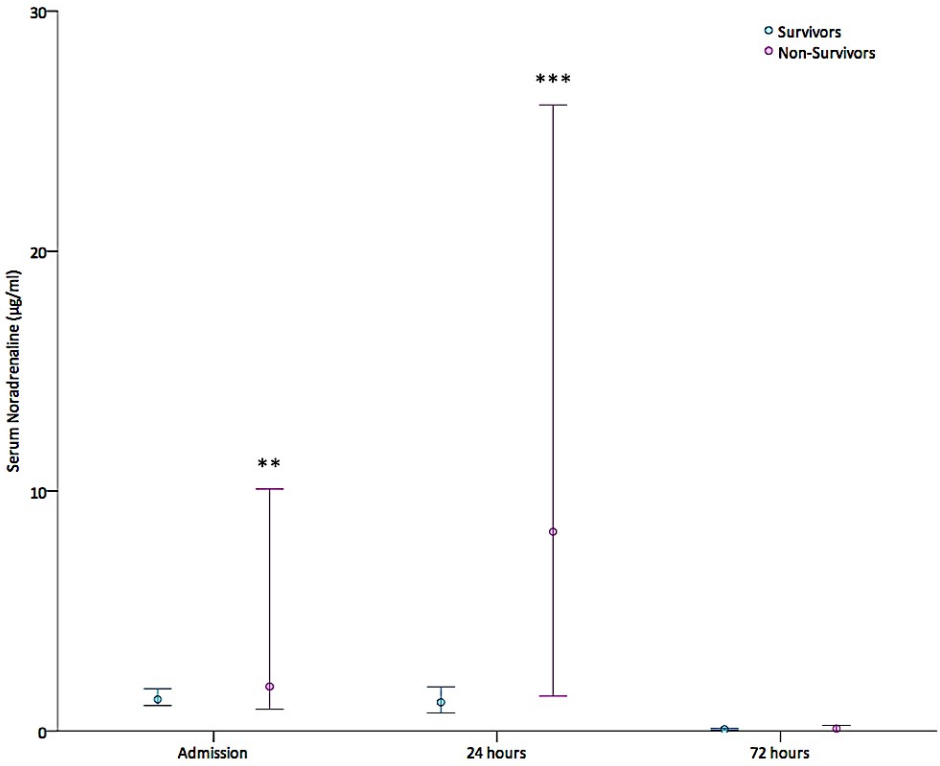


Figure 4.22 *Plot showing difference in noradrenaline levels in survivors and non-survivors- On admission median noradrenaline levels were 1.09µg/ml (IQR: 0.50-2.18) without inotrope use vs. 1.74µg/ml (IQR: 0.81- 4.02) with inotropes (p<0.02**). At 24h- 0.91µg/ml (IQR: 0.18-4.18) without inotropes vs. 8.08 (IQR: 1.67- 20.23) with inotropes (p<0.001***). At 72h- 0.07µg/ml (IQR: 0.04-0.13) without inotropes vs 0.1 µg/ml (IQR: 0- 0.18) with inotropes (p=0.9).*

Adrenaline levels were also significantly higher on admission, with plasma levels four times greater in the non-survivors at 1.12 µg/ml in comparison to 0.27 µg/ml in survivors. At 24 hours there was no significant difference between the two groups (0.18 µg/ml in non- survivors vs 0.10 µg/ml in survivors). At 72 hours, there was a second elevation/rebound in plasma adrenaline levels, the non-survivors having five times greater (4.54 µg/ml) compared to in survivors (0.91 µg/ml).

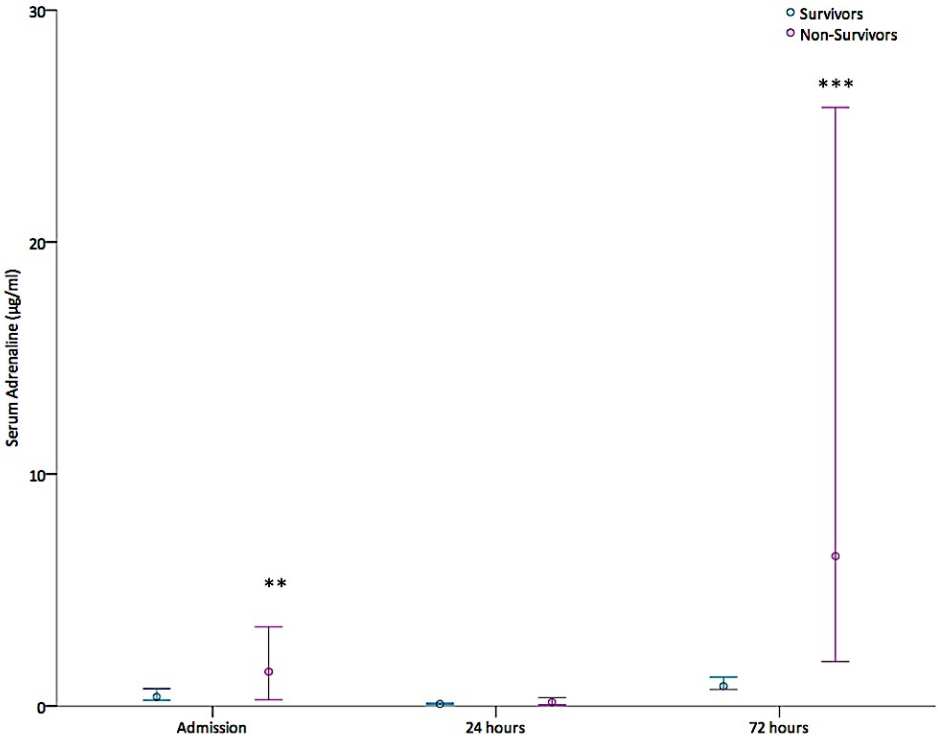


Figure 4.23 *Plot showing difference in adrenaline levels in survivors and non-survivors- On admission median levels were 0.002µg/ml (IQR: 0-0.009) without inotrope use vs. median 1.12µg/ml (IQR: 0.36- 3.21) with inotropes (p<0.02**). At 24h- 0.10µg/ml (IQR: 0.04- 0.21) without inotrope use vs. 0.18 (IQR: 0.06- 0.36) with inotropes (p=0.1). At 72h- 0.91µg/ml (IQR: 0.51- 2.30) without inotrope use vs. 4.34 µg/ml (IQR: 2.25- 9.05) with inotropes (p<0.001***).*

Dopamine levels peaked at 24h post admission and were declining at the 24h time point in both survivors and non-survivors. Non-survivors had elevated plasma dopamine at all time points, with a significant difference seen at 24h, with non survivors having three times higher plasma dopamine levels at 0.03 µg/ml when compared to 0.009 µg/ml in survivors. This elevation is also in keeping with increased prevalence of adverse cardiac events at 24 hours.

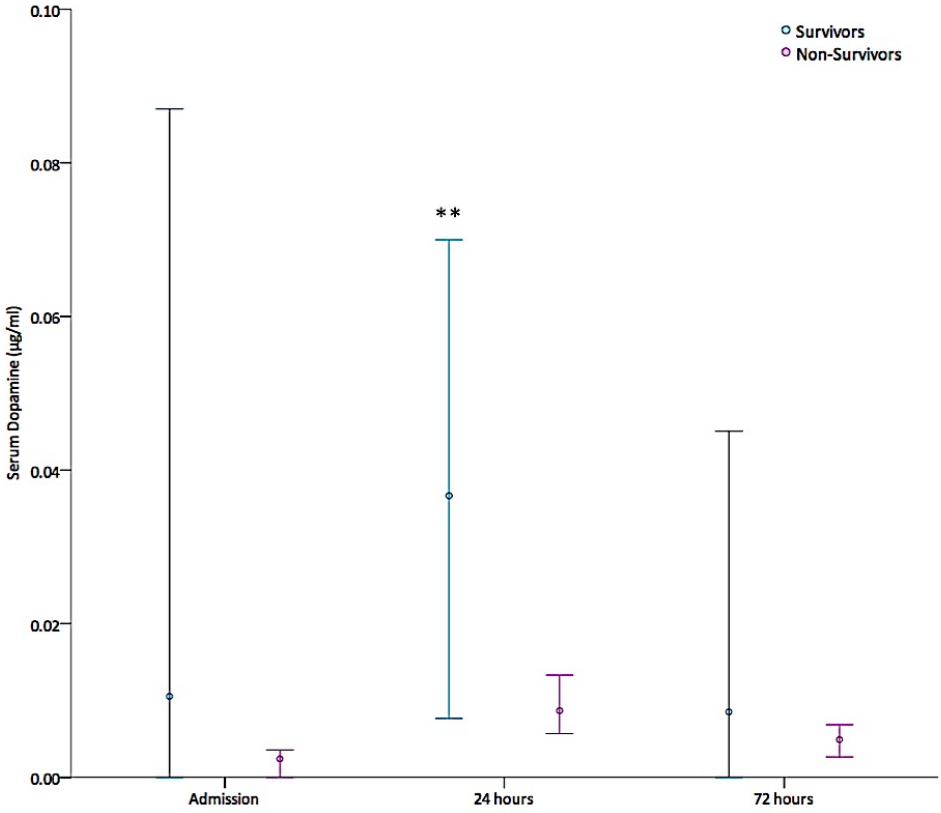


Figure 4.24 *Plot showing difference in dopamine levels in survivors and non-survivors- On admission median dopamine levels were 0.002µg/ml (IQR: 0-0.009) without inotrope use vs. 0.003µg/ml (IQR: 0- 0.03) with inotropes (p=0.1). At 24h- 0.010µg/ml (IQR: 0.004- 0.02) without inotropes vs. 0.03 (IQR: 0.008- 0.05) with inotropes (p=0.001**). At 72h- 0.004µg/ml (IQR: 0-0.009) without inotropes vs 0.009 µg/ml (IQR: 0- 0.04) with inotropes (p=0.2).*

4.3.8. Catecholamines in Predicting Adverse Cardiac Events

Binomial logistic regression was conducted including the variables listed in Table 4.2. The model had a -2 log likelihood of 71.3 ($p<0.001$), compared to the chi squared of 106.1 of the base model, therefore the inclusion of the variables improves the predictive value of the model. The pseudo R^2 value of 0.50 (Nagelkerke) suggest the model accounts for 50% of the variance in predicting ACE. In addition, the Hosmer and Lemeshow test of goodness of fit gave a p value of 0.936, therefore we do not reject the null hypothesis that the model is a good fit. Hence, this model can be considered a robust risk predictive tool. The output from SPSS are given below in Tables 4.2- 4.6.

Table 4.2 Omnibus Tests of Model Coefficients demonstrates a significant difference between the null model and the block model

		Chi-square	df	Sig.
Step 1	**Step**	71.296	15	.000
	Block	71.296	15	.000
	Model	71.296	15	.000

Table 4.3 Summary of the model

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	106.075[a]	.282	.502

Table 4.4 Hosmer and Lemeshow Test of goodness of fit

Step	Chi-square	df	Sig.
1	2.974	8	.936

Table 4.5 Classification table demonstrating the predictive value of null model and model with variables

Observed			Predicted		
			ACE		
			No ACE	ACE	% Correct
Step 0	**ACE**	**No ACE**	202	0	100.0
		ACE	36	0	.0
	Overall %				84.9
Step 1	**ACE**	**No ACE**	177	7	96.2
		ACE	16	15	48.4
	Overall %				89.3

Table 4.6 Variables included in the model.

						95% C.I.for EXP(B)	
	B	S.E.	Wald	Sig.	Exp(B)	Lower	Upper
Sex	.103	.803	.016	.898	1.108	.230	5.342
Age	.016	.017	.854	.355	1.016	.982	1.051
ISS	.016	.029	.320	.571	1.016	.961	1.075
Thorax AIS	.361	.192	3.530	.060	1.435	.985	2.090
H&N AIS	.321	.182	3.090	.079	1.378	.964	1.970
IHD	.763	.968	.621	.431	2.145	.321	14.311
Diabetes	-2.163	1.247	3.011	.083	.115	.010	1.324
Hypertension	2.131	.765	7.768	.005	8.424	1.882	37.698
Hyperchol	1.195	.963	1.539	.215	3.303	.500	21.813
HR	.006	.010	.367	.545	1.006	.986	1.027
SBP	-.016	.007	4.696	.030	.984	.971	.999
Lactate	.194	.096	4.094	.043	1.214	1.006	1.466
Noradrenaline	.007	.013	.273	.601	1.007	.981	1.033
Adrenaline	.041	.164	.061	.804	1.042	.755	1.437
Dopamine	3.470	3.719	.871	.351	32.142	.022	47068.853
Constant	-4.728	2.053	5.301	.021	.009		

B is the co-efficient of variable in the model, with associated standard error. The Wald chi-square and its two tailed p value are given in testing the null hypothesis that B=0. Exp(B) is the odds ratio for each variable with the 95% confidence interval.

Within this model hypertension, Systolic blood pressure and lactate were identified as independent factors in the development of adverse cardiac events. According to the odds ratio from the model, pre-existing hypertension leads to 8 fold higher risk of developing an adverse cardiac event. Hypertension may affect adverse outcomes in many ways. The hypertensive patients are predisposed to underlying endothelial damage and coronary artery disease, which in turn may lead to adverse cardiac events with increased physiological stress (143). Hypertension also predisposes to the development of atrial fibrillation, which may be precipitated by the injury (144). An alternative explanation may be that patients with pre-existing hypertension and potentially left ventricular hypertrophy have poorer ability to autoregulate their coronary perfusion and even with minimal hypotension, there is a reduction in cardiac perfusion leading to myocardial ischaemia and therefore injury (145). Lactate and systolic blood pressure are both indicative of hypoperfusion leading to potential cardiac injury.

Within this model, thoracic injury does not significantly contribute to the development of ACE, therefore suggesting that it is not related to direct chest injury. Furthermore, this model also negates the contribution of catecholamine exposure on admission to the development of adverse cardiac event. On examining the correlation matrix there was no significant co-linearity within the variables, suggesting that within this model the inotropes levels recorded in this cohort do not contribute to the development of ACE.

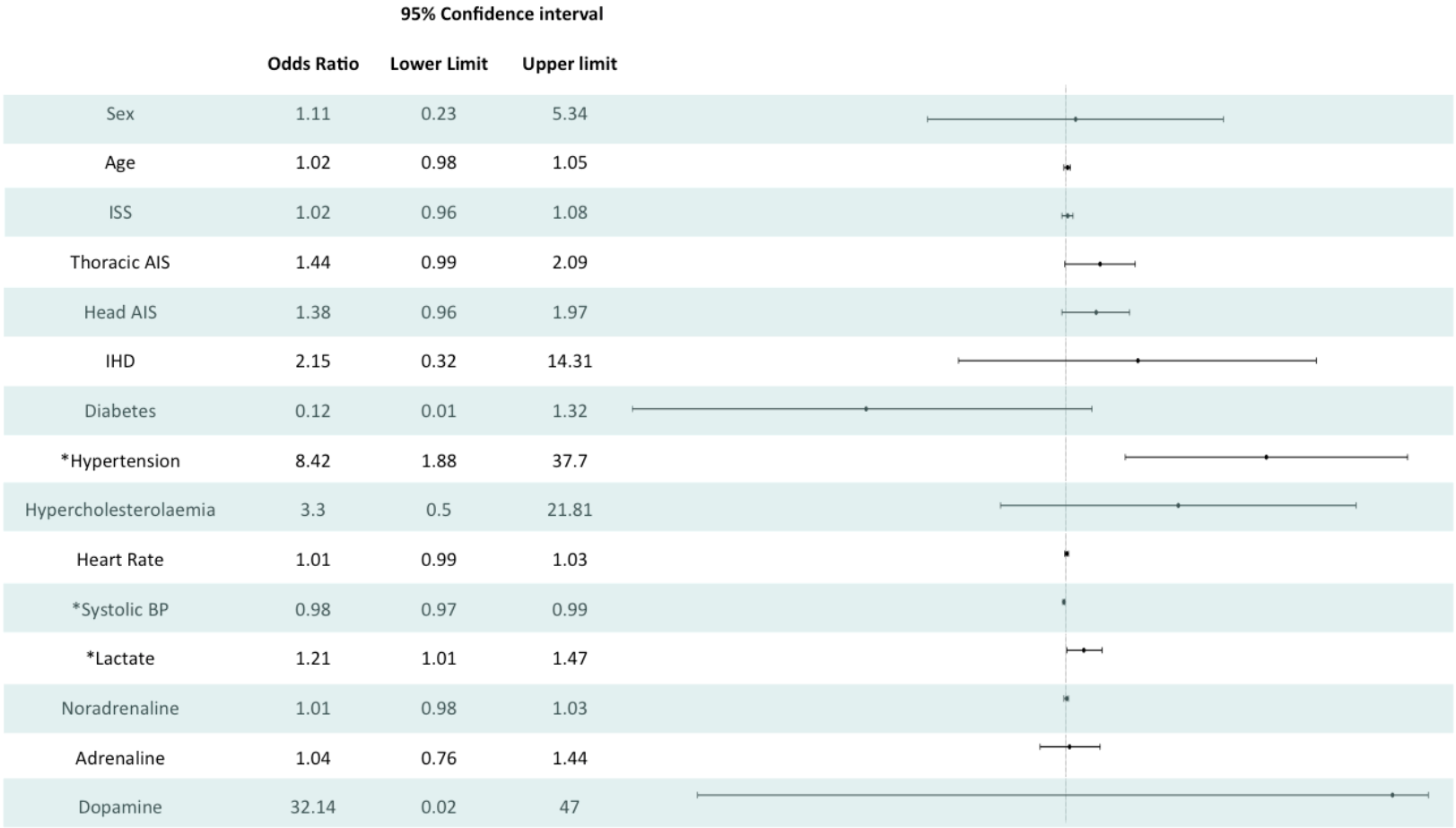


Figure 4.5: Forest plot showing the odds ratio of admission variables for the development of ACE

4.4 Summary of Chapter

Beta-adrenergic blockade has been identified as having potential benefits in critically ill trauma patients based primarily on retrospective cohort studies of pre-injury beta-blocker use. There are a number of putative mechanisms for catecholamine-induced cardiac injury, including ischemia due to poor diastolic filling, or directly-induced calcium overload and cardiomyocyte necrosis (95).

In our study, admission levels of plasma adrenaline and noradrenaline were significantly higher in the patients who developed ACE. Furthermore, increasing levels of adrenaline and noradrenaline were associated with increasing levels of h-FABP on admission and 24 hours after injury. Despite this, we were unable to statistically separate any differential effects of adrenergic stimulation from general shock and hypoperfusion. While h-FABP levels were elevated on admission as shown in the previous chapter they continue to rise over the first 24 hours. There is therefore the potential to modulate on going cardiac injury.

At the point of admission, all patients in this cohort had not received any exogenous catecholamine and therefore the plasma levels recorded on admission could be purely attributed to endogenous production.

Measurement of catecholamines can be difficult to perform and analyse due to their short half-life. However, the biomarker analysis employed here was

determined to be the most practical way of measuring catecholamines in trauma patients.

In summary, adrenaline and noradrenaline levels were associated with TISCI, the development of ACEs and subsequent outcomes including mortality. The exact role of catecholamines in the genesis of TISCI remains unclear and further clinical and experimental work is warranted. There exists an opportunity to improve outcomes for these patients with patient stratification and future targeted therapy. Finally, as mentioned, with this cohort study we were unable to specifically determine whether TISCI is purely a catecholamine-induced phenomenon. This should be further validated with animal models.

Chapter 5

Cellular mechanisms of Trauma Induced Secondary Cardiac Injury

5.1. Introduction

Traumatic injury and haemorrhage, imposes elevated state of physiological stress. The bleeding combined with tissue injury leads to a varying concoction of hypoperfusion, coagulopathy, catecholamine excess, release of DAMPs and immunological disarray which poses a huge risk to cellular processes(146). The myocardium with its usual high oxygen demands and a delicate blood supply is prone to damage during this catastrophic state. The sustained effort required to maintain perfusion in the face of haemorrhage, alongside a multitude of noxious assaults, exerts pressure directly on the cardiomyocytes. Clinical studies have shown the cardiac dysfunction in trauma haemorrhage can be evidenced by clinical events, ECG changes and an increase in circulating biomarkers(118,147). The cardiac dysfunction has been replicated in animal models with cytokines, inflammatory pathways and apoptosis implicated in the pathogenesis (148). However, in the studies looking at apoptosis and cardiac dysfunction, surprisingly, there was no increase in cardiac biomarkers (104).

Trauma haemorrhage is not a distinct pathology. It is a cumulative syndrome resulting from bleeding, tissue injury, direct organ injury, immune dysregulation and a systemic inflammatory response (146). The simulation of this condition in animal models, manipulation of the control factors and isolation of organs help further our understanding. Animal models of trauma haemorrhage have been honed and standardised over years (148). Dr Johanna Wall was able to produce these models in our group and furthermore demonstrate a rise in h-FABP as seen in our patients,

providing a murine model of TISCI. The release of cardiac proteins into the bloodstream is thought to be due to disruption of the cardiomyocte cell membrane as a result of ischaemic insult (35). However, due to its abundance as a free cytosolic protein and its small size, h-FABP may be released during early ischaemia (65).

5.2 Study Aims

This study aims to understand the nature and severity of cellular changes that lead to the release of cardiac proteins upon trauma injury and haemorrhage as seen in the clinical studies. Cell membrane disruption is a key feature of cellular stress and augural of cell death. Myocardial death is not a reversible injury and will leave patients with prolonged morbidity (106). Increasing our understanding of the pattern of injury and the causal mechanisms may help improve patient outcomes in the future.

The work in this chapter aims to further investigate the effect of trauma haemorrhage specifically on the cardiomyocyte. Western blotting and histology were used to study structural and biochemical changes that may shed further light on the the cellular pathways that underlie myocardial cell death following traumatic injury and haemorrhage .

Initially, through histopathological examination, the gross changes of the myocardium in TISCI were explored, TEM allowed for further visualization of the

ultrastructure structure of the heart. Subsequently, western blot analysis was used to further interrogate changes in cellular levels of biomarkers and pathways that may be driving the structural changes

5.2 Results

5.2.1 Ultrastructural Changes In The Trauma Haemorrhage Models

The rise in serum cardiac proteins along with their reflective depletion from the myocardial tissue indicates disruption of cell membrane integrity and subsequent cell death. In myocardial ischaemia, necrosis is often associated with the ischaemic changes, therefore transmission electron microscopy (TEM) images of the heart were studied to identify ultrastructural changes in this TH mice model, that may reveal the underlying processes.

The sham animals demonstrated normal myocardium with contracted myofibrils with clearly demonstrable A bands and Z discs (Figure 5.1). The nuclear chromatin is evenly distributed with a dense nucleoplasm with surrounding glycogen. The mitochondria are evenly distributed alongside the sacromere. The mitochondria are smooth edged with evenly dense matrix scattered with matrix granules. The cristae are intact and tightly packed (149) (150). Overall, 23% of the mitochondria demonstrate dense bodies, however, there are no associated features of ischaemia surrounding these mitochondria.

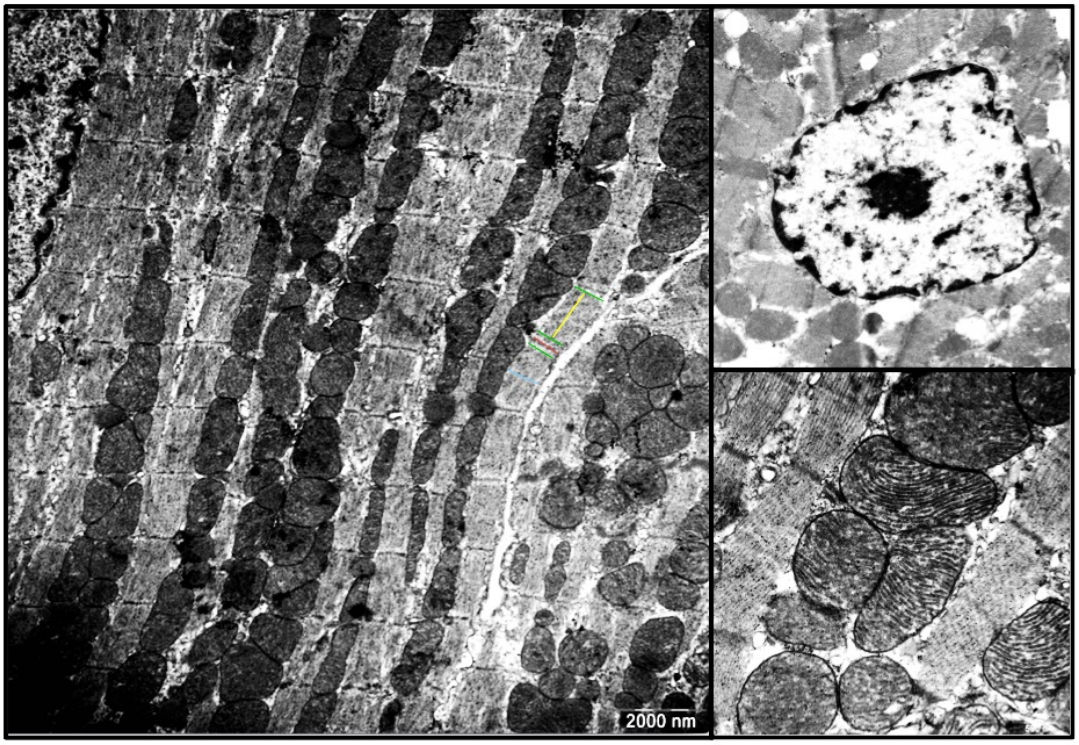


Figure 5.1: *Transmission Electron Microscopy (TEM) of myocardium (left) from sham mice, showing well-organised myofibrils clearly define with A bands (green) and Z discs (red), M band (blue) and I bands. The nucleus (top right) shows a regular intact nuclear envelope with evenly distributed chromatin in the nucleoplasm. The mitochondria (bottom right) are evenly distributed along the sarcoplasm with clearly defined cristae and even electron density within the matrix.*

There are varying degrees of severity in the changes seen within the heart of the same animal. The organelles in different regions of the same heart demonstrate microscopic changes in keeping with reversible as well as irreversible cellular injury.

In areas with mild injury, the cardiac tissue maintains its similarity to the sham animals. These regions have well defined sarcomeres with linear myofibrils. However, the I bands are more prominent, indicative of relaxation of the muscle, which has been shown to be associated with global ischaemia (150). There is also evidence of dispersed interstitial oedema with associated mitochondrial oedema as evidenced by the loss of electron density. In previous studies of murine models of

cardiac ischaemia, oedematous changes were demonstrated within 30 minutes of coronary artery ligation (149). In these less affected regions, the mitochondria remain linearly distributed and retain normality in their appearance. The cristae are intact and tightly packed with evenly dense matrix and the presence of matrix granules. However, the mitochondria in these regions do demonstrate amorphous dense bodies.

In the TH mice, 33% of the mitochondria contain amorphous dense bodies in comparison to 23% in the sham animals (p=0.006). Although, the loss of intra-mitochondrial granularity has been described as one of the earliest changes in ischaemia, in these models and preceded by dense body formation. The nuclei within these regions show no signs of adverse changes.

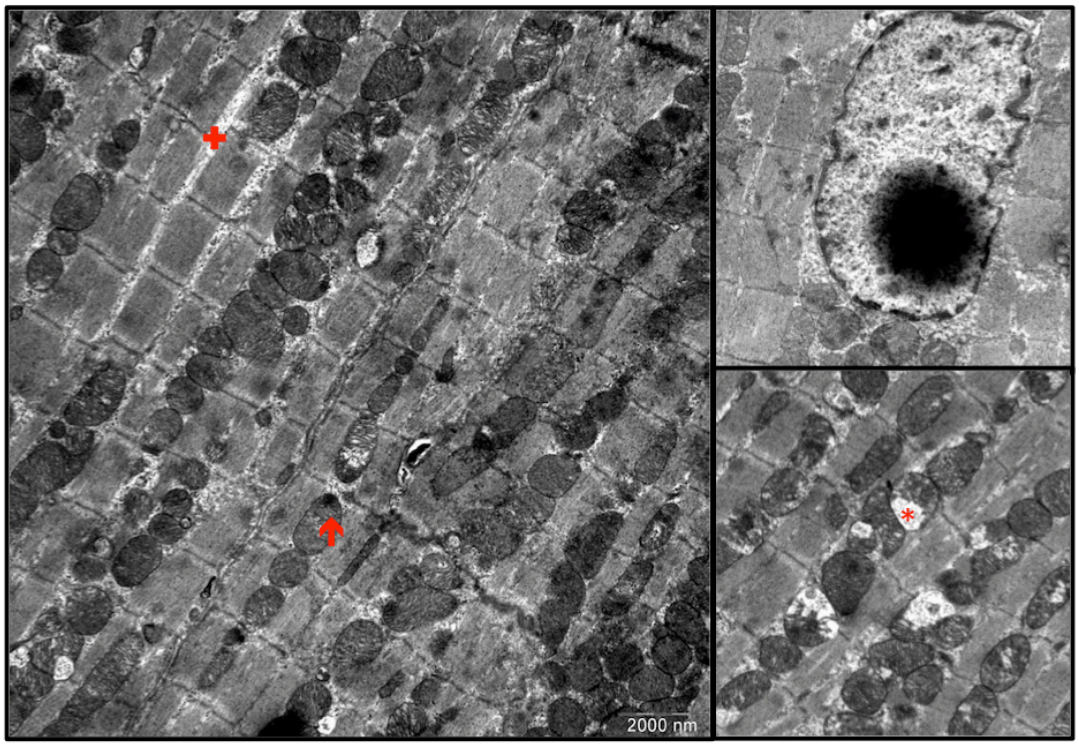


Figure 5.2: *TH model myocardium (left) demonstrate interstitial (cross) and mitochondrial oedema (bottom right- asterik). The mitochondria contained dense bodies (left- arrow) however the nuclei remain normal (top right)*

In areas with more significant damage, the organelles lose their structural definition. There is diffuse interstitial oedema present with associated myofibrillar disruption and autolysis. This may be indicative of necrosis. The mitochondria also demonstrate signs of sustained hypoxia such as mitochondrial swelling, with loss of electron dense material from the matrix, breakdown of the cristae and vacuolation. Rarefaction of the nucleoplasm, where there is loss of the chromatin as demonstrated in these models, is often seen when ischaemia extends greater than 15 minutes (151). The nuclei in our TH animals also exhibit signs of irreversible structural changes, for example margination of chromatin, associated with surrounding oedema. Nuclei with chromatin condensation are contained in an organised fashion within an oraganelle like structure, which is neither in keeping with disruption of cell membrane seen with necrosis nor the blebbing of apoptosomes (152).

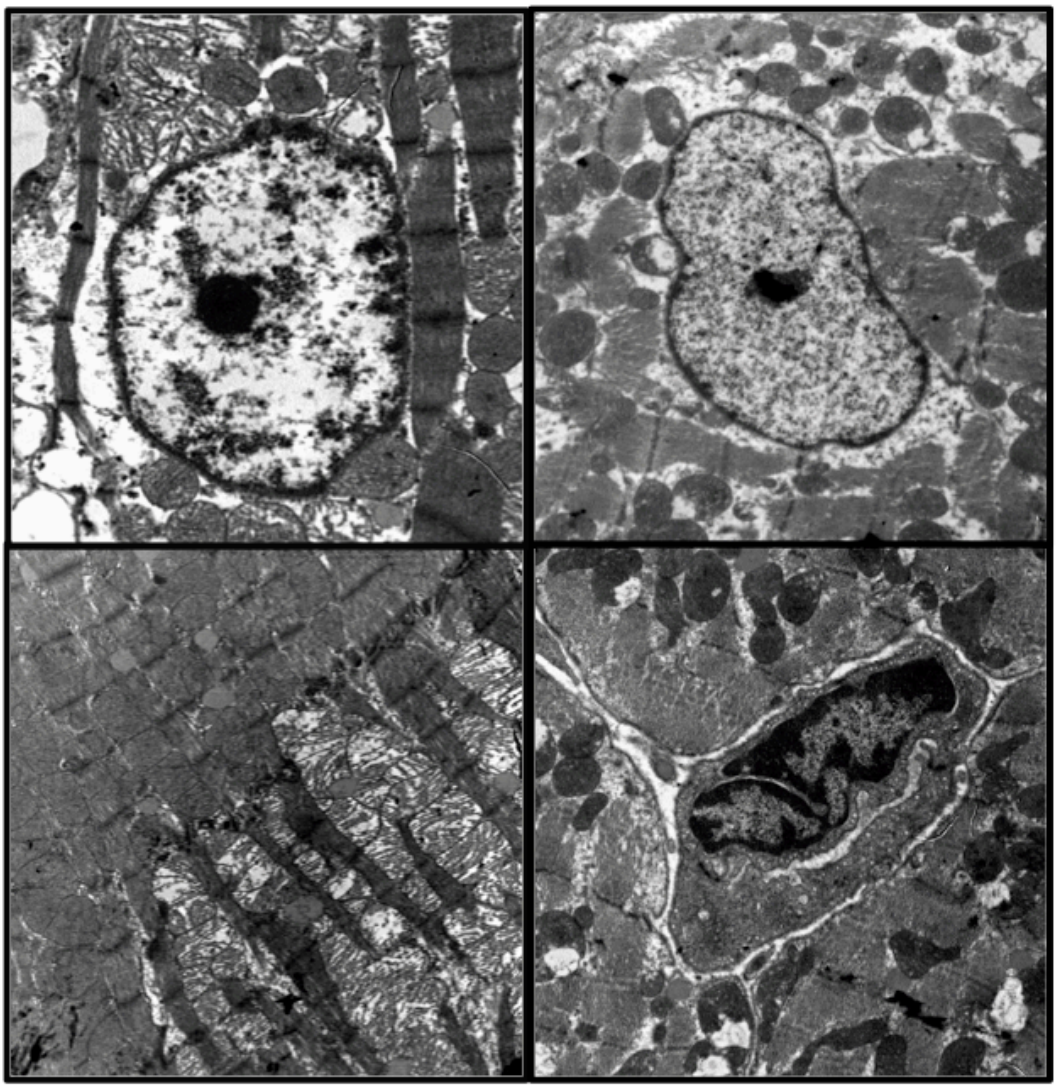


Figure 5.3: *Areas of severe cellular injury with rarefaction of the nucleoplasm (top) with surrounding oedema (top left) and glycogen depletion (top right). There are also significant amounts of mitochondrial oedema (bottom left) seen throughout. Nucleus (bottom right) seen with margination of chromatin within a well contained dead cell in TH model cardiomyocytes.*

In two in the longest surviving animals, the sarcomere partly retains its ultrastructure with the definition of the myofibrils, however they are fully relaxed with visible contraction bands. The extracellular oedema is associated with widened intercalated discs and lifting of the sarcolemma with widened intercalated discs. There is interstitial oedema with disruption of the linearity of the sarcomeres with mitochondria dispersed in a disorganised fashion. There are multiple vacuoles

and lipid deposition dispersed throughout the sarcomere. There is also rarefaction of the nucleoplasm with margination of chromatin in the nucleus. The mitochondria are fragmented and irregular in shape, raising the possibility of mitochondrial fission. The mitochondrial matrix demonstrates increased electron density with poorly defined cristae. The distribution of the mitochondria is not sustained across the sarcomeres with surrounding vacuolisation.

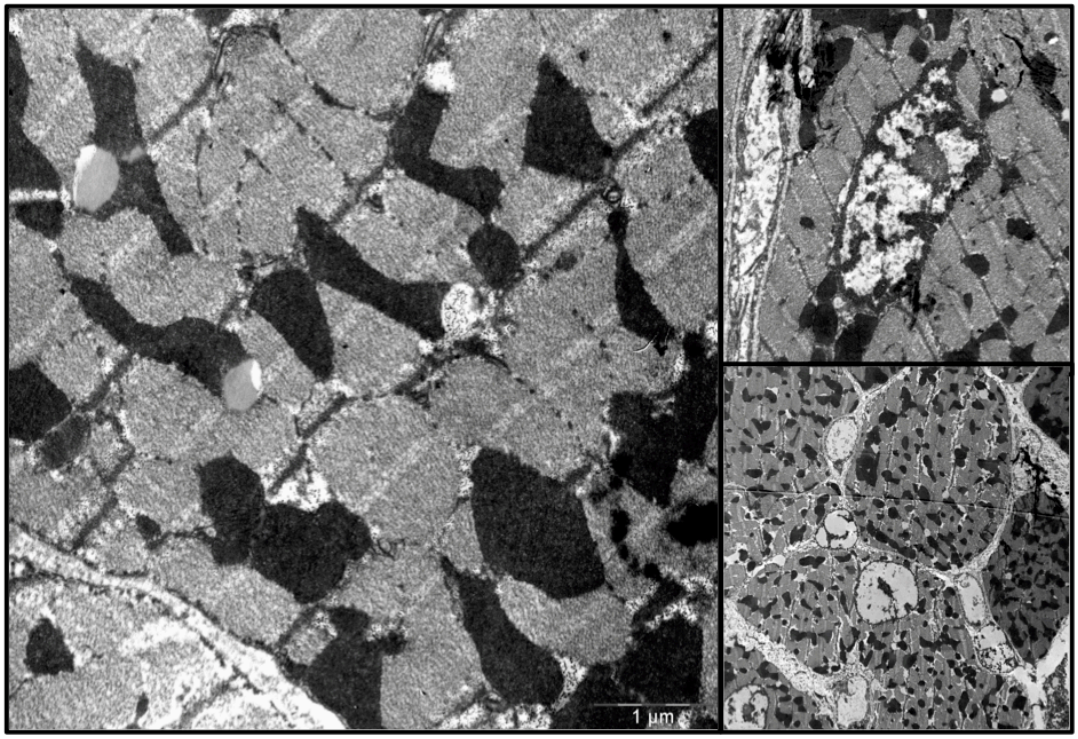


Figure 5.4: *TH model myocardial tissue displays widespread disorganistion of the myocardium and relaxation of the sarcomere demonstrating prominent M & I bands (left) and poorly circumscribed mitochondria distributed in a disorderly fashion. The nuclei (top right) shows margination of chromatin and rarefaction of the nucleoplasm. There is widespread interstitial oedema (bottom right) with vacuolation and lipid deposition.*

Overall, electron microscopy of the TH mouse hearts demonstrate significant changes within the myocardium consistent with presence of myocardial ischaemia in line.

5.2.2 Quantifying Protein Expression In The Myocardium

The TH murine model demonstrates cardiac injury through an increase in serum troponin and h-FABP (Methods 2.3). Western blot analysis and immunohistochemistry were used to examine if these increases in serum proteins had concurrent effects on the intracellular protein expression and distribution within the cardiac tissue.

Paraffin sections from the hearts of 8 sham animals and 6 resuscitated TH models were used for immunohistochemistry analysis of protein expression. In the sham animals 3 points of interest were analysed on 4 separate sections and in the TH models 4-5 points of interest were studied from 2 different sections. The single colour images were transformed to pixelated images using Image-J software to allow for approximate quantification of the proteins of interest and expression reported as mean fluorescence.

Protein extraction from the hearts of the trauma haemorrhage model was subjected SDS PAGE gel electrophoresis and blotted on PVDF membrane with specific antibodies for analyzing target protein expression analysis. A silver stain was then used to standardise proteins of interests. This was repeated for the protein extracted from mitochondrial fractions isolated from mouse tissue (Methods 2.4).

5.2.2.1 Troponin-I Expression in Cardiac Tissue

A cardiac tissue specific, mouse monoclonal cTnI antibody was used for identification of cTnI. The antibody specifically detects the full length of the cardiac cTnI protein, by targeting the epitope spanning 23 to 29 amino acids.

On gross examination, there were no obvious changes in the pattern of cTnI distribution upon IHC examination (Figure 5.6). However, following the initial overview of the protein distribution, image-j analysis demonstrated a reduction in the mean fluorescence of cTnI in the TH hearts when compared to the sham hearts, 17.1 and 12.2 respectively, p=0.004. The loss of cTnI proteins was also observed in the TH hearts using western blotting. The optical density for the TH animals was 0.1 compared to 0.06 in the sham animals. However, the difference was not significantly different when the intensity optical density of the blot signal was quantified. The downregulation of the expression of troponin I in the TH animals as demonstrated by immunohistochemistry and western blotting is in keeping with the rise in serum biomarkers seen in these animal models.

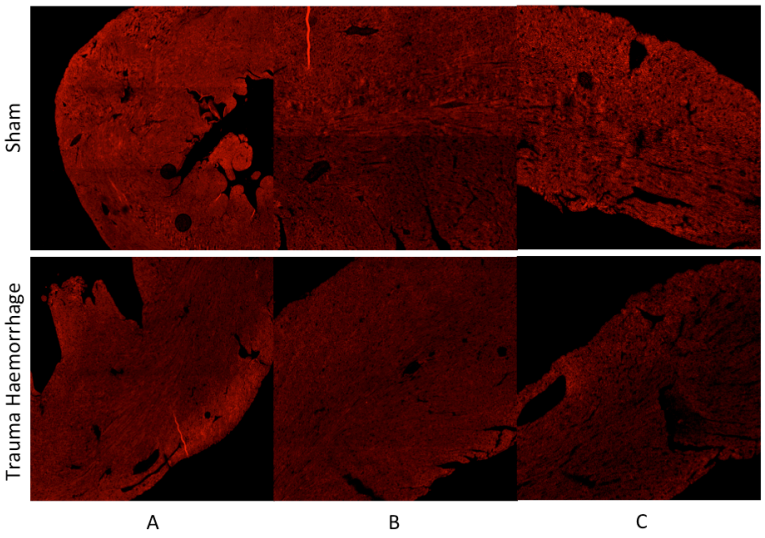


Figure 5.5: Troponin expression as demonstrated by IHC of paraffin embedded cardiac tissue. *The sections were stained with primary mouse monoclonal cTnI antibody and conjugated with AF594. Section was scanned using tiling programme at 20 x magnification. Top- sham operated animal (n=8) and bottom- TH model (n=6). A- Left ventricle, B- Interventricular wall and C- Right ventricle*

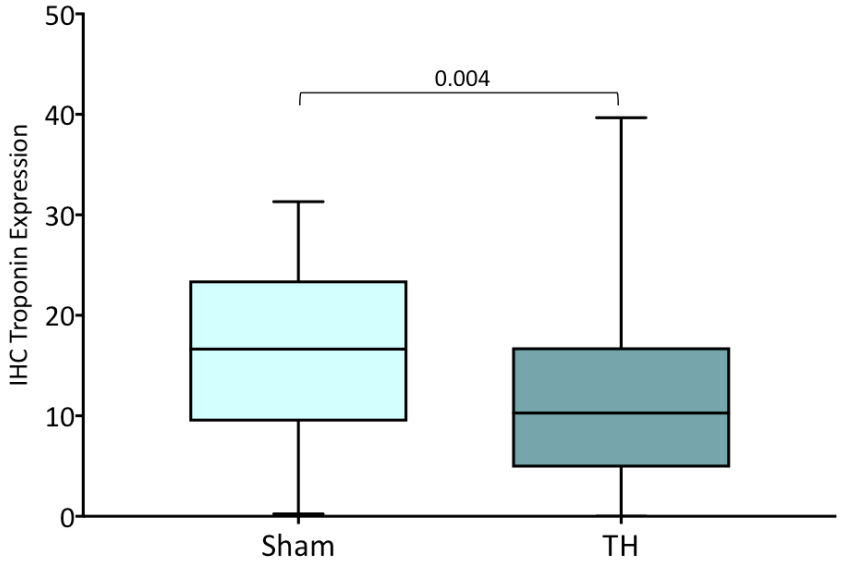


Figure 5.6: Troponin expression derived from IHC *of paraffin embedded cardiac tissue. Mean fluorescence 17.1 in sham-operated animal (n=8) and 12.2 in TH animals (n=6), p=0.004*

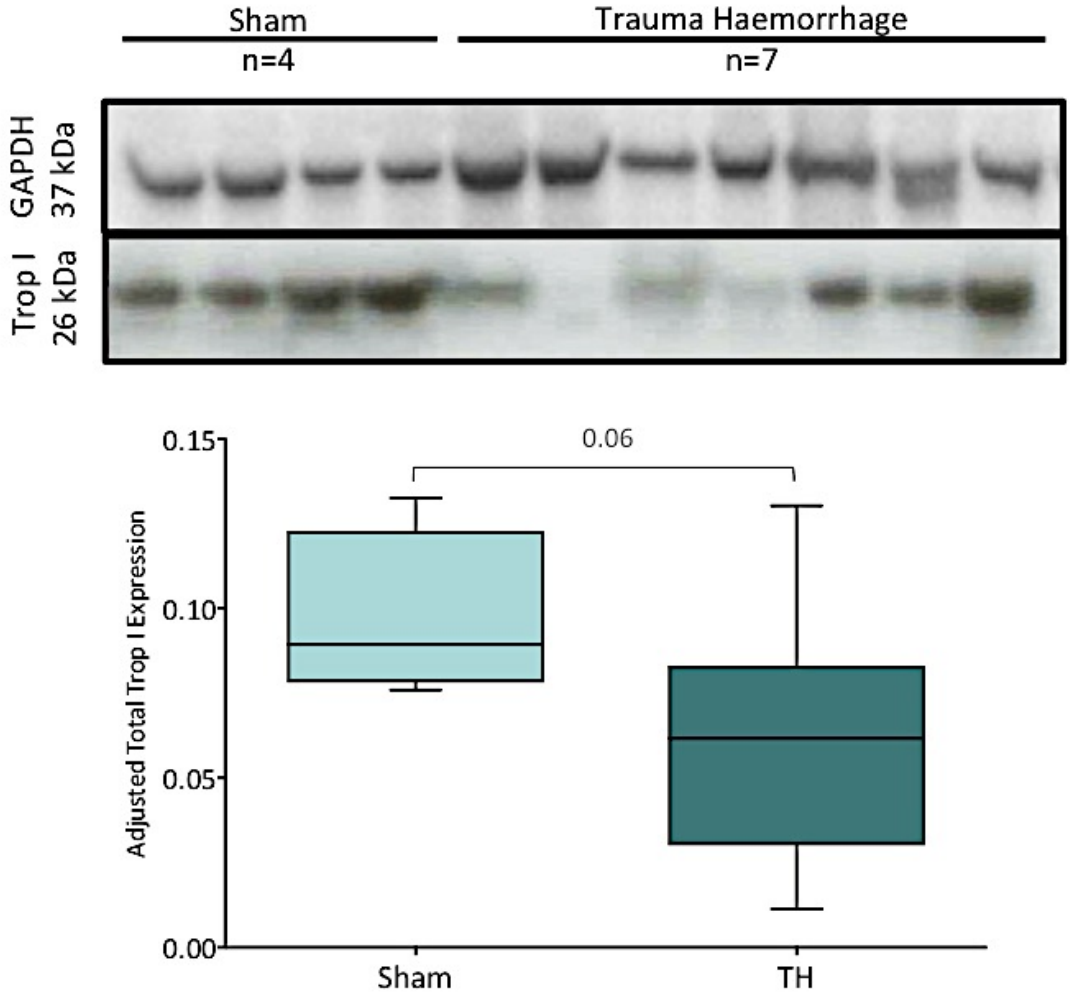


Figure 5.7*: *Troponin expression as demonstrated by western blot analysis. *Adjusted mean intensity of 0.10 in sham (n=4) and 0.06 trauma haemorrhage (n=7), p=0.06*

5.2.2.2 H-FABP Expression In Cardiac Tissue

Alterations in cellular H-FABP protein expression were investigated through western blotting using specific antibodies. The expression of h-FABP in the TH mice was significantly lower when compared to the sham animals, 0.05 vs. 0.03 (p=0.04).

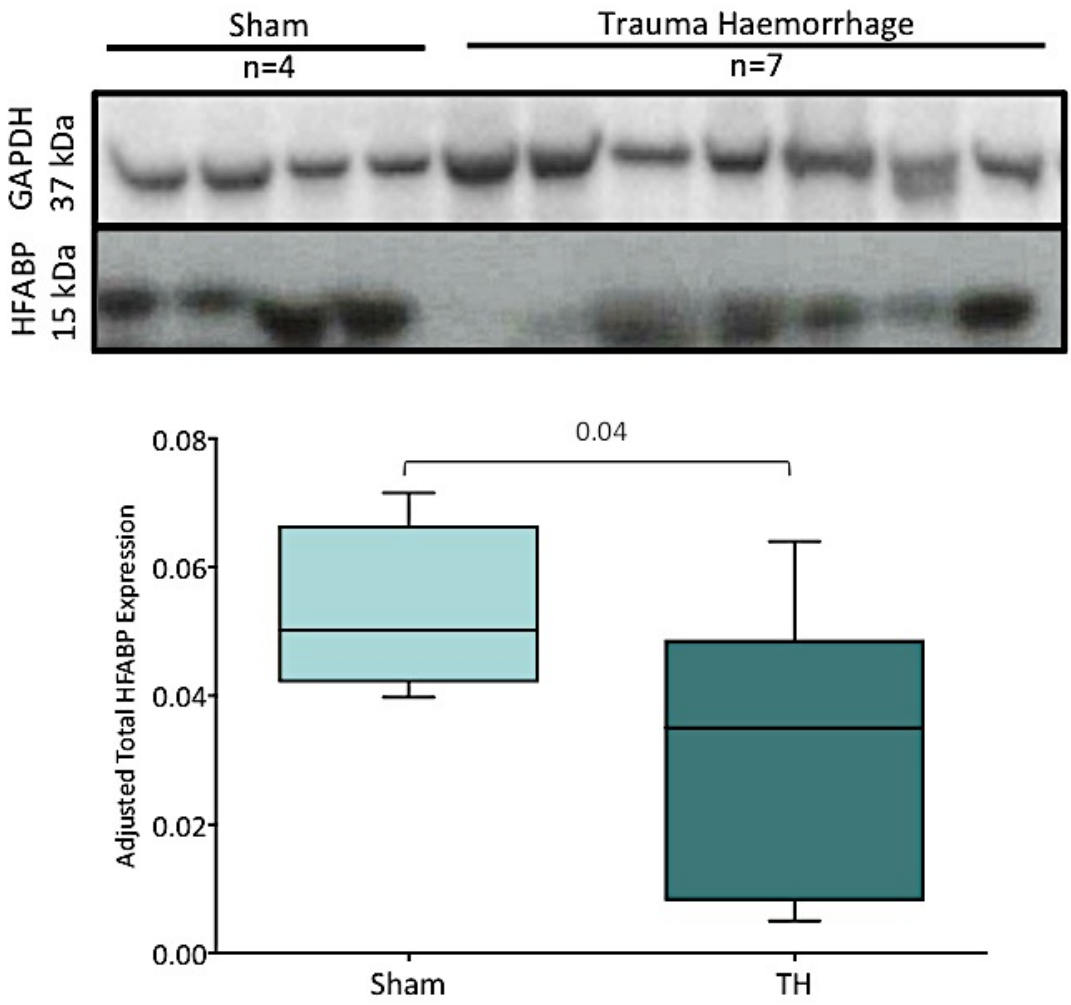


Figure 5.8: Western blot of h-FABP expression. *Adjusted optical density is 0.05 in sham-operated animals (n=8) and 0.03 in trauma haemorrhage (n-=6) murine hearts, p=0.04.*

5.2.3 Signalling Pathways to Cell Death

The decrease in cardiac structural protein expression as well as the associated ultrastructural changes indicate the onset of cell death (153). Traditionally, cell death has been defined into distinct processes such as necrosis, apoptosis, autophagy and oncosis. However, with newer insights, it is becoming more apparent that there is a degree of crossover between the pathways (106). Although, a rise in cardiac biomarkers is contributed to myocardial necrosis and cell membrane disruption, previous studies have associated cardiac dysfunction in trauma with increased myocardial apoptosis (35,104). The diversity in the cellular changes seen on EM with the absence of profound autolysis suggests that there may be many drivers of cell death in these models and not all dying cells are condemned to the same eventuality. To identify the cell death mechanisms that lead to these changes, downstream proteins of the extrinsic and intrinsic cell death pathways were examined.

5.2.3.1 Caspase 8 Expression

Caspase 8 is a downstream effector of the death receptor initiated cell death pathway. Upon western blotting using specific antibody and after adjusting the intensity of bands expression of Caspase 8, revealed similar, 0.07 vs. 0.06 ($p=0.20$) levels of caspase expression in the samples of TH and the sham models. However, IHC revealed significant down regulation caspase-8 expression, 18.69 vs. 1.6 ($p=0.001$).

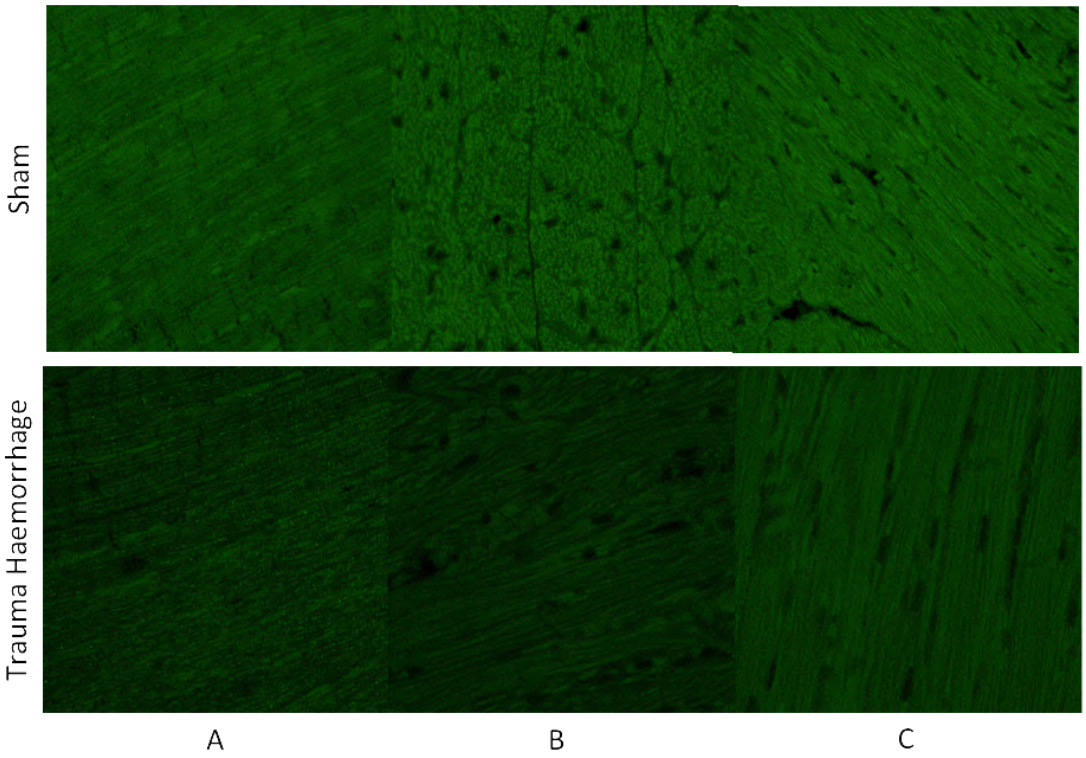


Figure 5.9: *IHC of Caspase 8 expression IHC of paraffin embedded cardiac tissue stained for caspase 8 showing reduced immunofluorescence in the TH mice hearts when compared to the sham animals. Each section presented in the figures are from a separate animal.*

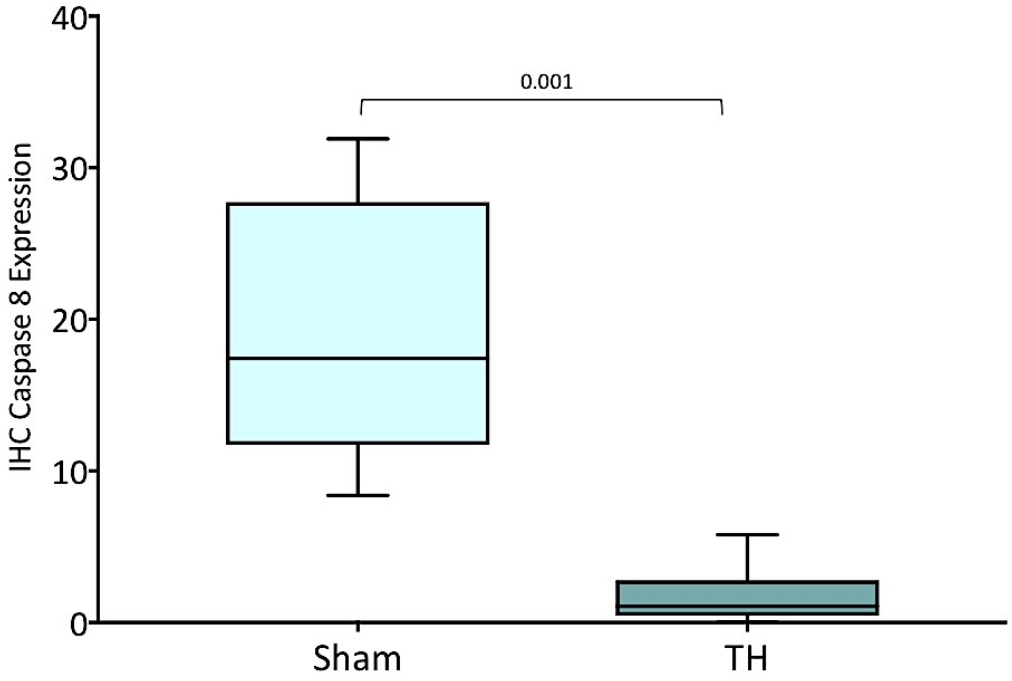


Figure 5.10 *Caspase 8 expression derived from IHC of paraffin embedded cardiac tissue. Mean fluorescence 18.7 in sham-operated animal (n=8) and 1.6 inTH animals (n=6), p=0.004.*

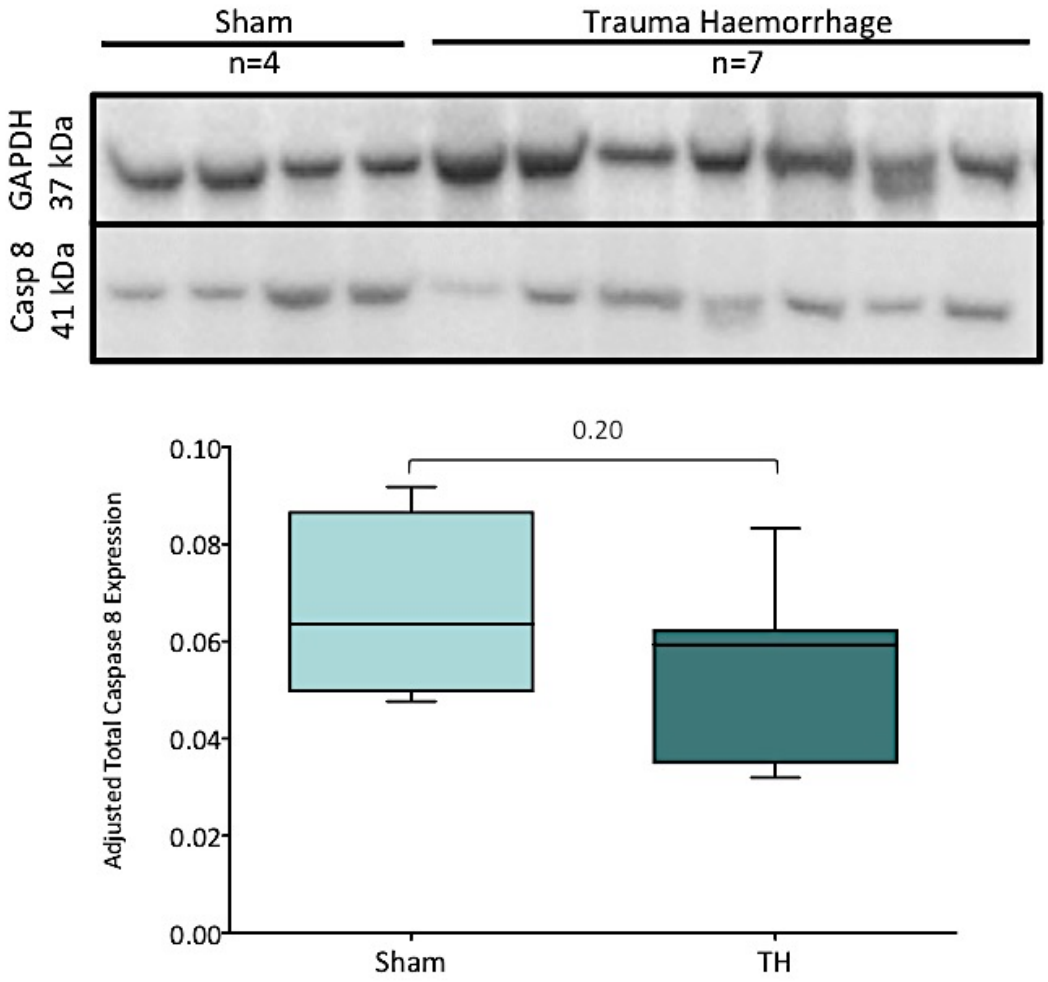


Figure 5.11 *Western blots of Caspase 8 expression. Optical density in sham-operated animals is 0.07 compared to 0.06 in trauma haemorrhage animals, p=0.20*

5.2.3.2 Expression and Localisation of AIF

The activation of mitochondrial driven apoptosis is dependent on the influx of cell death proteins from within the mitochondria into the cytosol. Upon exposure to toxins such as damaged DNA or ROS, PARP-1 receptors are activated leading to the subsequent activation of Bcl pathways which in turn results in the permeabilisation of the outer mitochondrial membrane (154). This permeabilisation leads to the cleavage of the apoptosis inducing factor (AIF) to its death-inducing active form into the cytosol, which then translocates to the nucleus where it leads to DNA degradation (110). Furthermore, the breakdown of the mitochondrial membrane allows the release of cytochrome-C. Free cytochrome C within the cytosol of the cell is then able to induce further cell death pathways through activation of caspase-3 dependent apoptosis ((106)).

To explore this, fresh murine hearts were homogenized and mitochondria were separated to look at the differential distribution of AIF and cytochrome C. In addition to the whole cell expression of AIF protein, cells were fractionated into the cytosol and mitochondria portions in order to investigate alterations of AIF compartmentalisation following TH.

Western blotting analysis demonstrated that the protein expression levels of AIF were comparable in both sham and trauma haemorrhage animals with mean densities of 0.20 vs. 0.17 ($p=0.50$).

To further elucidate the distribution of proteins in the different sub cellular fractions obtained from heart tissue of TH models, the subcellular fractionation procedures are followed and differential distribution of specific proteins of interest, between the cytosol and mitochondria were investigated. Heart tissue was homogenized and then processed by differential centrifugation; at low speed to remove large organelles and debris and using a specific mitochondrial extraction buffer (see methods section 2.4.3) by high-speed centrifugation to isolate the mitochondria. The fractionated samples were run through the gels and probed for AIF and positive controls were used to confirm mitochondrial separation- MTCO2 as a mitochondrial marker and GAPDH as a cytosolic marker. The change in distribution of AIF in the mitochondria versus the cytosol was assessed. The subcellular fractionation procedure removes the nuclei from the supernatant and therefore, further analysis was done to compare the mitochondrial expression of AIF against the total expression.

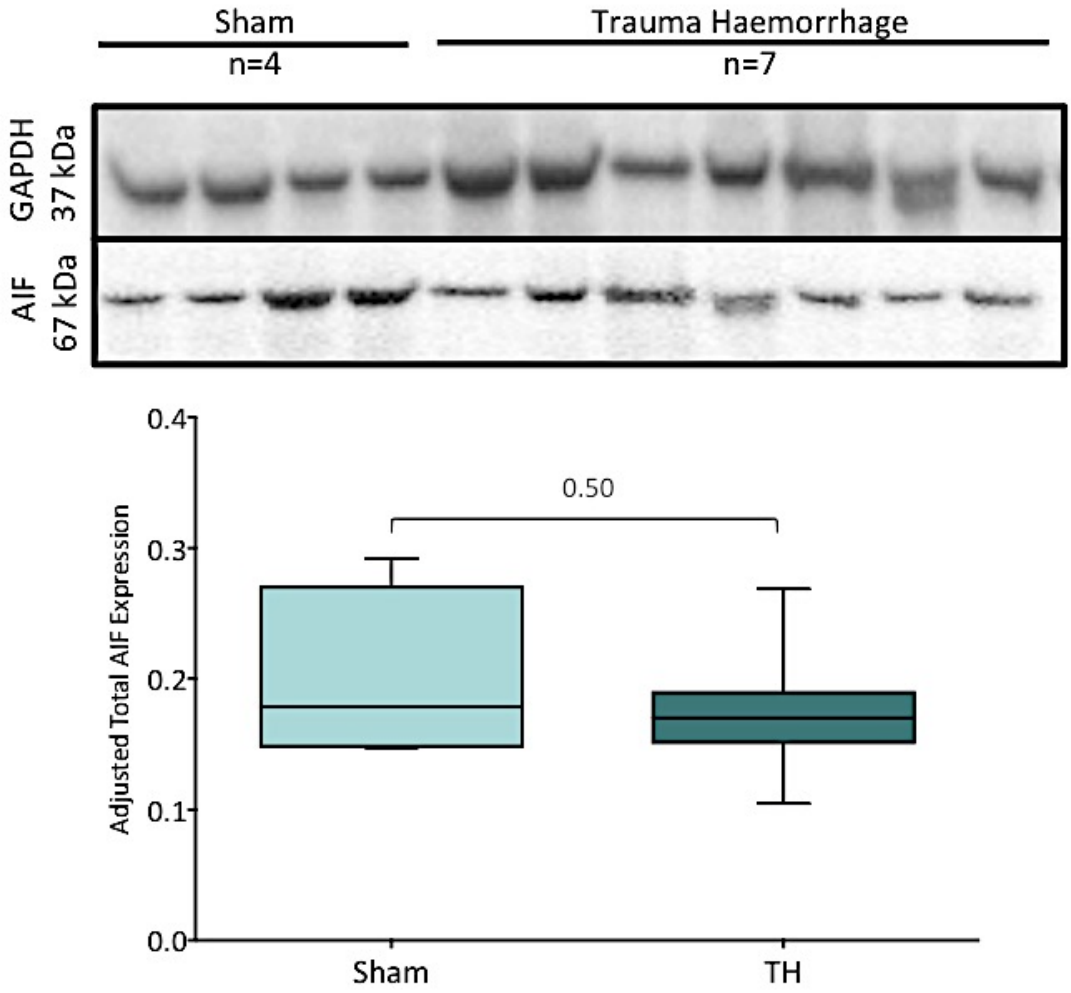


Figure 5.12: AIF expression as demonstrated by western blot analysis. Mean optical density for sham group was 0.20 vs 0.17 for the TH group

In comparison to the sham animals, the TH animals show a reduction in AIF distribution in the mitochondria to supernatant ratio. The mean ratio in the sham models is 1.5 in comparison to 0.4 in the TH models, however this is not statistically significant. This is suggestive that there is an increase in release of AIF in to the supernatant where the trauma becomes lethal in these mice. When the ratios are studied as the distribution between the mitochondria and total cell content that include the nuclei, the difference in AIF expression becomes statistically different. The mean ratio in the sham models is 0.6 when compared to 0.3 in the TH models

(p=0.04). This is suggestive of mitochondrial AIF leaching into cytosol and potentially its translocation to the nucleus.

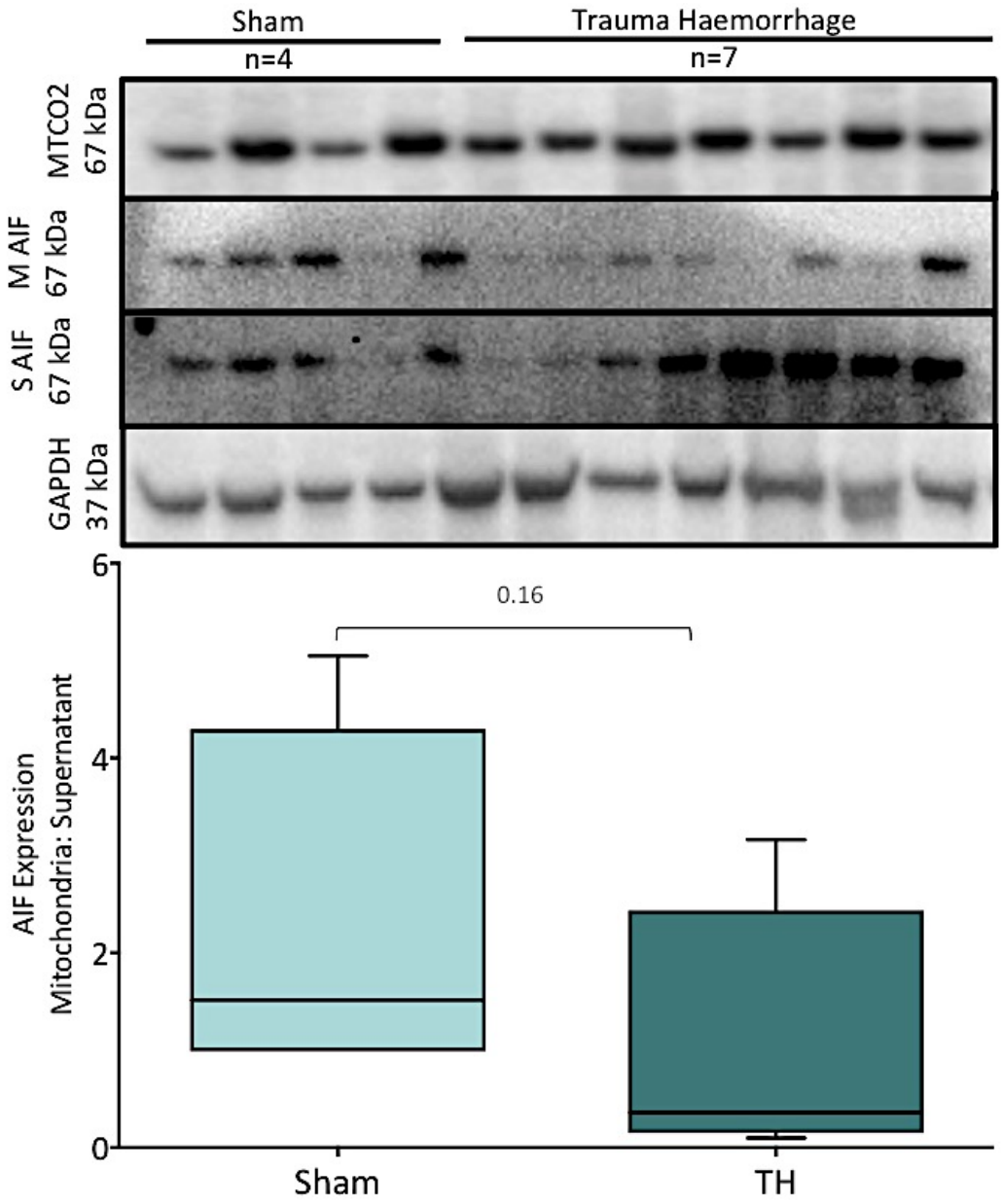


Figure 5.13: Comparison of mitochondrial vs Supernatant AIF compartmentalisation *in sham and trauma haemorrhage murine hearts as demonstrated by western blots. The mean ratio of mitochondrial AIF (M AIF) to supernatant AIF (S AIF) is 1.5 in sham group compared to 0.4 in TH group, p=0.16.*

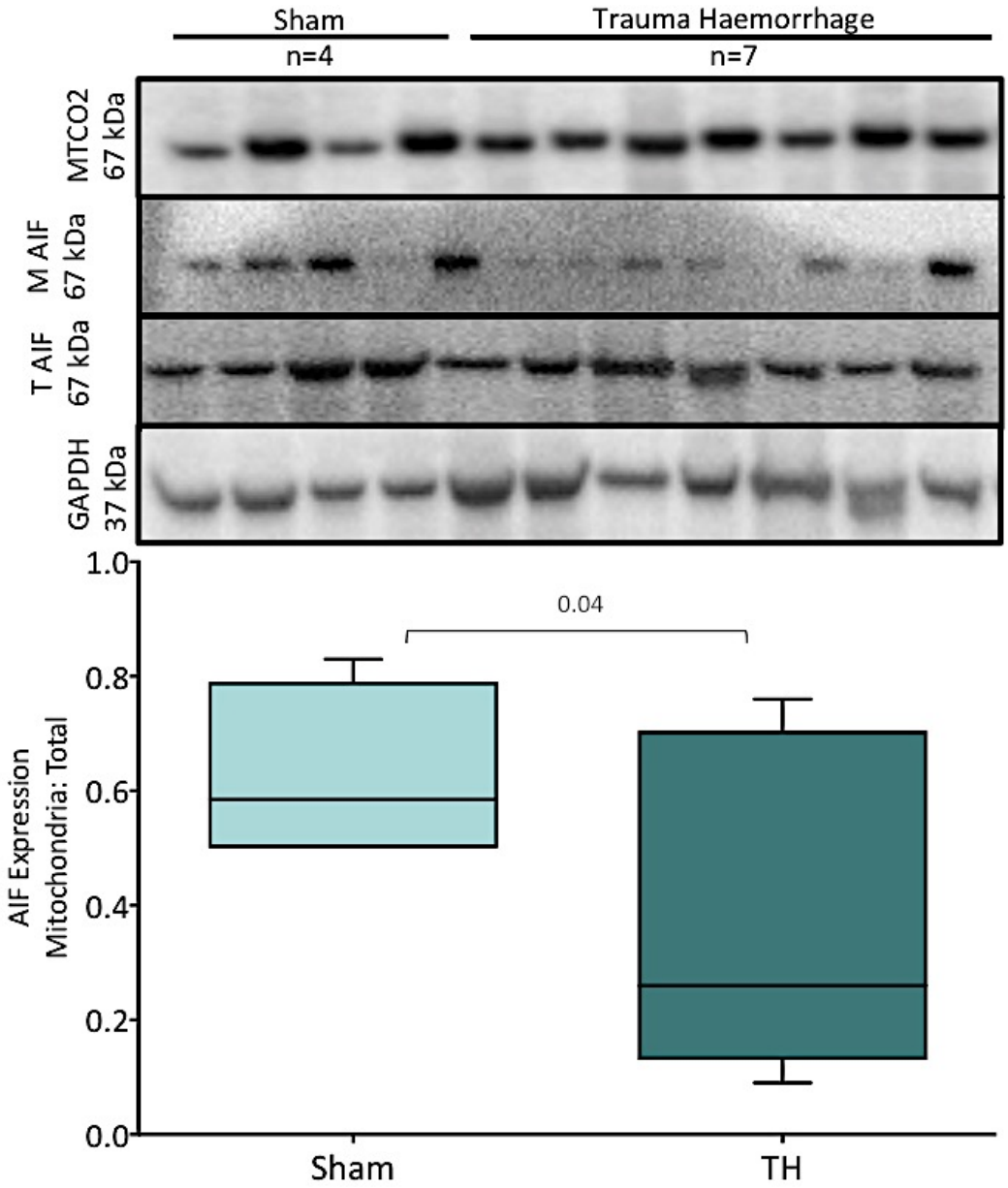


Figure 5.14: Comparison of AIF expression in whole tissue and in the mitochondria *of sham and trauma haemorrhage murine hearts as demonstrated by western blots. The ratio of total AIF (T AIF) to mitochondrial AIF (M AIF) in sham operated group is 0.6 compared to 0.3 in TH animals, p=0.04.*

Immunofluorescent microscopy was used to visualise the localisation of the cell death associated protein AIF with concurrent staining of a mitochondrial marker, MTCO2. The nucleus was directly stained with DAPI (blue). MTCO2 was a primary mouse antibody, that was further incubated with an anti-mouse secondary labeled

with AF 594 (red) and AIF was an anti-rabbit primary antibody that was further labeled with an anti-rabbit secondary antibody with AF 488 label (green).

AIF expression was examined through immunohistochemistry. In contrast to the expression measured by western blot, this demonstrated an increase in AIF expression in the TH models. The mean immunofluorescence exhibited by AIF in the TH models was double that of the sham animals 13 vs. 6 (p0.001).

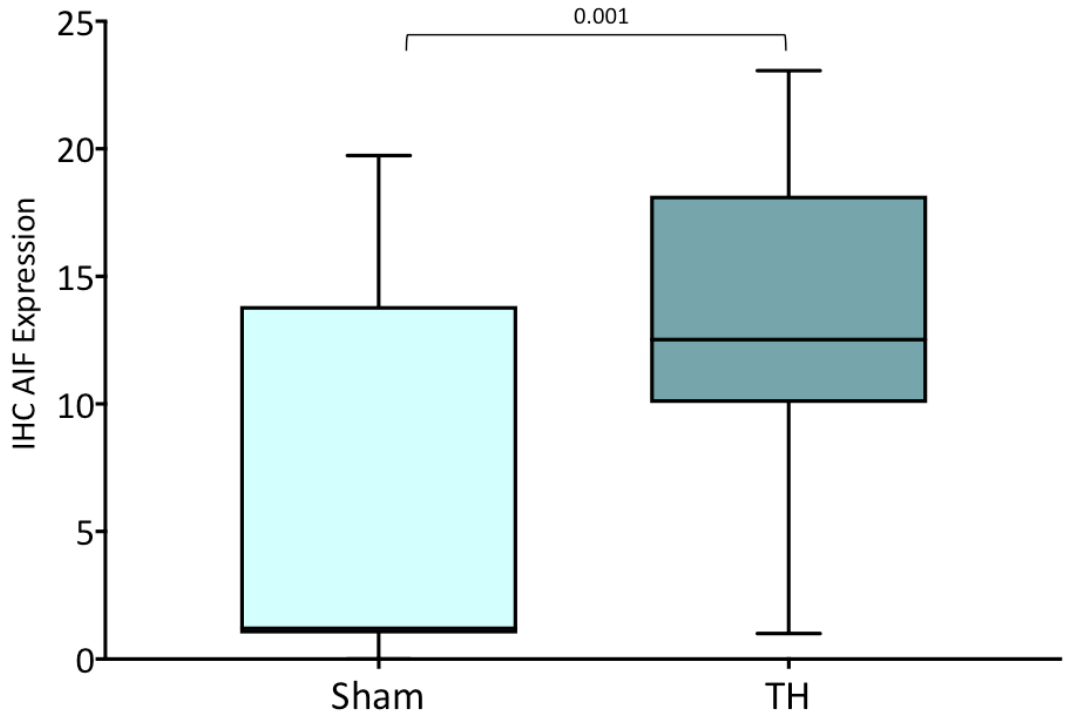
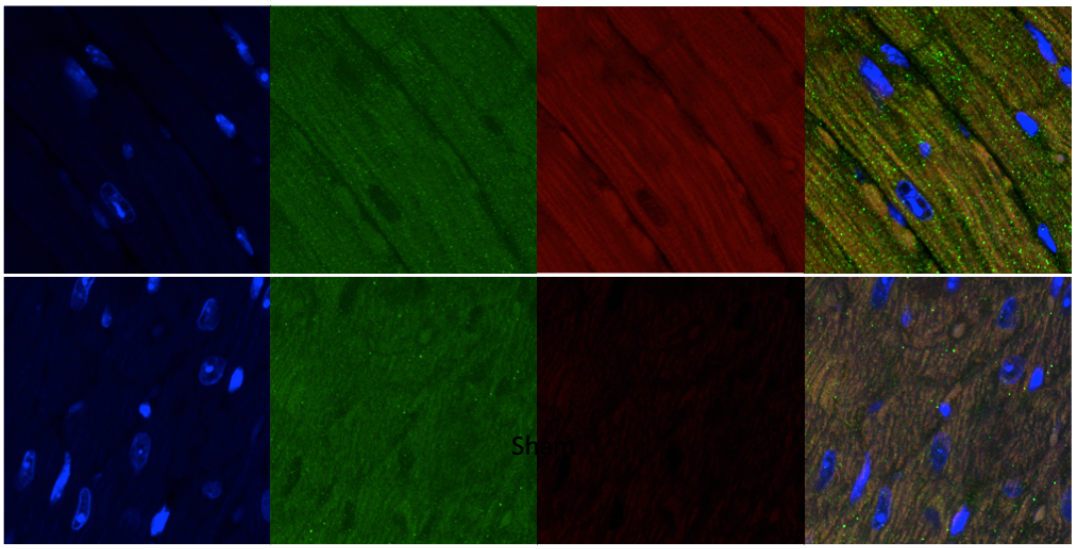


Figure 5.15: AIF expression as measured by immunofluorescence *in paraffin sections. The mean fluorescence was calculated to be 13 in sham animals vs. 6 in the TH animals, p=0.001*

On IHC analysis the nuclear localisation of AIF was more apparent in the resuscitated TH models when compared to the Sham models (Figure 5.16). The image-j software was utilised to account for the degree of co-localisation of the proteins. Mitochondrial localisation was calculated to be 0.05 in the TH models compared to 0.18 in the sham, however this was not statistically significant. Whereas, the difference in nuclear localization was statistically different with a mean of 0 in the sham and 0.06 in the TH models.



Figu.re 5.16: *Sections from sham-operated animals, DAPI (blue), AIF (green), MTCO2 (red) and combined images*

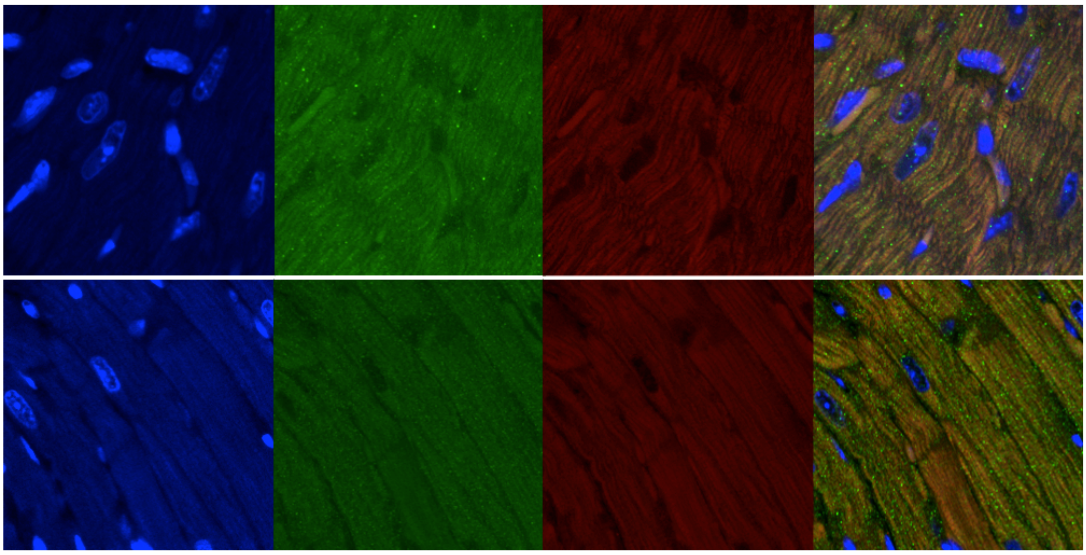


Figure 5.17: *Paraffin embedded sections from TH animals, DAPI (blue), AIF (green), MTCO2 (red) and combined images. In the combined images, localisation of AIF in the nucleus can be seen*

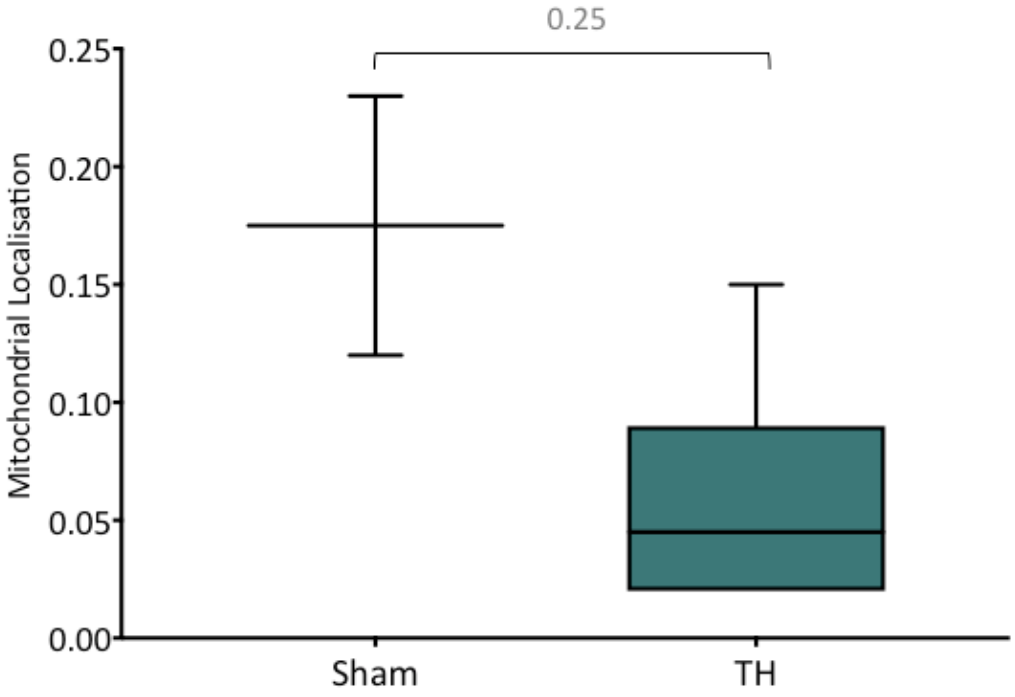


Figure 5.18: *Mitochondrial Localisation of AIF as calculated by Image-J in sham animals 0.18 in comparison to 0.05 in TH animals, p=0.25*

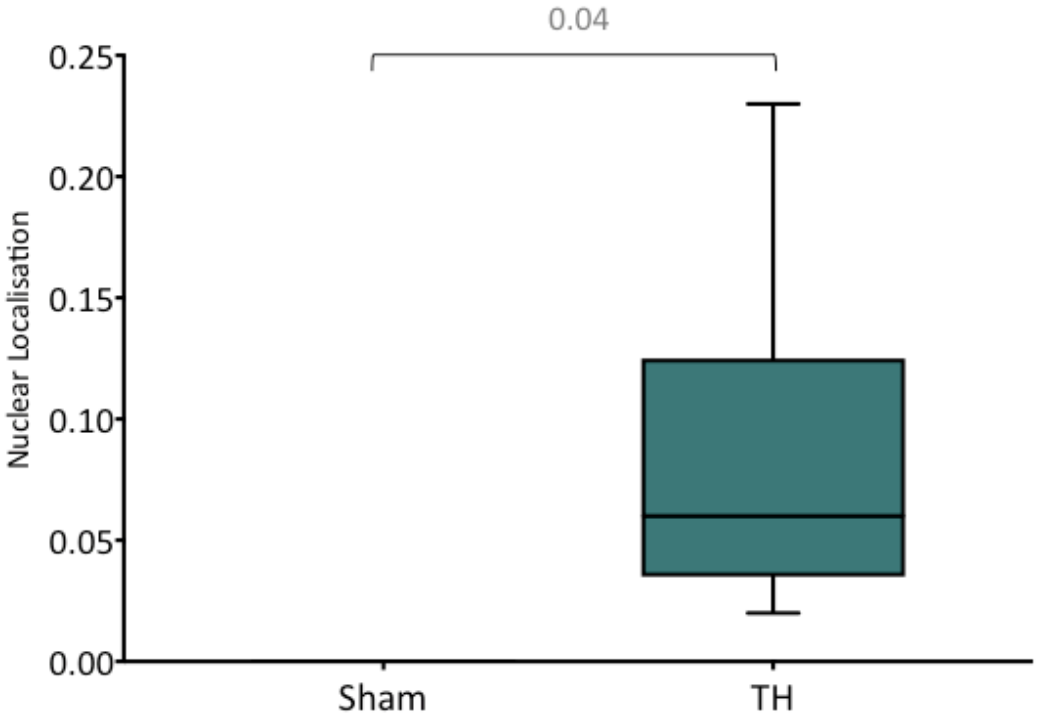


Figure 5.19: *Nuclear Localisation of AIF as calculated by Image-J in sham animals 0 in comparison to 0.06 in TH animals, p=0.04*

5.3 Summary Of Chapter

The aim of this chapter of work was to further understand the mechanisms that lead to ACE and the release of cardiac biomarkers. Using a mouse model of TH allowed the harvest of heart tissues in order to visualise gross and ultrastructural changes in the architecture of the tissue. Furthermore, it allowed probing of several proteins thought to be associated with the cardiac stress response as demonstrated in chapter 3 and 4. This enabled a better understanding of the underlying changes associated with the rise in cardiac biomarkers and ultra-structural anomalies in the subcellular components such as the mitochondria in the murine trauma haemorrhage models and potential drivers for the responses seen in our trauma patients.

In the sham animals, EM demonstrated normal myocardial structure with well-defined myofibrils, defined sarcoplasm and organized mitochondria throughout. Whereas in TH mice this normal architecture and cellular organisation appeared disrupted. The most prominent feature in the myocardium of TH mice was the cellular oedema, varying from interspersed interstitial oedema to gross oedema leading to organelle swelling and myofibrillar disruption. Structural changes in mitochondria are markers of cell stress and these mitochondria fail in efficient energy production. This occurs due to lack of the Na+/K+ pump functioning in the outer membrane of mitochondria, and therefore leading to cytosolic accumulation of Na+ and Cl- ions (155). Furthermore, under hypoxic stress, anaerobic respiration,

leads to lactic acidosis and increased hydrogen ion production. This directly alters the function of the Na+/K+ pump precipitating the damage (155).

Mitochondrial amorphous dense bodies was present in both sham and TH models. There is a lot of variation in the significance of the amorphous dense bodies. Isolated myocardial cells have been noted to develop amorphous dense bodies with just incubation at 37^{o}C without any change in their function, thought to be secondary to reorganisation of the structural proteins (155). However it has also been associated with irreversible injury with changes in the mitochondria's ability to utilise pyruvate and effectively respire (151,156,157). The intra-mitochondrial dense bodies occur following accumulation of precipitated mitochondrial protein associated with calcium phosphate and influx of intracellular calcium, leading to ischaemia (158). However, dense bodies have been noted in sudden calcium influx is also associated with hyperadrenergic states, (151). Dense body formation has also been demonstrated following anaesthesia with Propofol in murine models due to the reduced coronary flow following anaesthesia-induced hypotension (159). The isoflurane similarly leads to hypotension, so its potential contribution to inducing dense body formation should be taken into account.

The TH mice demonstrated areas with severe injury, with widespread disorganisation of the myocardium and relaxed sarcomere in keeping with irreversible cell damage. These areas also demonstrated hollow mitochondrial matrix with increased electron density. Some of these changes may be in keeping with reperfusion injury due to sustained hypoxia followed by reflow due to

crystalloid resuscitation. Cells that are irreversibly damaged with disruption to cellular and mitochondrial membrane integrity have been shown to develop further swelling and destruction with the presence of vacuolation as seen here (149). Reperfusion of the cardiac tissue induces calcium influx into the cells perpetuating cellular damage in vulnerable cells which would be in keeping with the mitochondrial changes (149).

Serum troponin and h-FABP has been shown to be raised in trauma patients with associated adverse cardiac outcomes (Li2015, bahloul, (135,160). This has been reflected in our animal models with TH associated with rise in troponin and h-FABP. In this study, using the TH models of cardiac injury, we were able to establish a link between corresponding cellular changes and associated changes in protein expression. The western blots and immunohistochemistry studies demonstrated that these changes had reflective reduction in these cardiac proteins within the cardiac tissue. Cardiac troponin has been used in murine models of ischaemia induced by the ligation of coronary arteries (161). The induced infarct has been correlated with a rise in troponin alongside a reduction in cardiac function (161). The rise in troponin has also been demonstrated in models following isoproterenol induced cardiac toxicity (162). In these models, the rise in serum troponin was comparable to the degree of coagulative necrosis seen on histology. In TH models, a few studies have explored the role of cell death in cardiac dysfunction in animal models(104,163). The pro-apoptotic proteins were seen to rise with cardiac dysfunction without concurrent rise in plasma cardiac biomarkers, where the authors suggested the dysfunction was due inflammatory mediated and not due to

myocardial necrosis (104). However, as demonstrated by clinical studies, it has been noted that there is a rise in cardiac proteins such as troponin and h-FAPB. In our study we have demonstrated that this is associated with changes in keeping with ischaemia in the myocardium. To distinguish the cell death pathways involved in inducing myocardial cell damage and the leakage of proteins, caspase 8, AIF and cytochrome C were chosen. In our models there was no significant difference in the caspase 8 or cytochrome C expression as measured by western blot between sham and TH.

However, AIF a cell death factor that activates upon mitochondrial stress is observed to translocate into nucleus from mitochondrion as observed in our subcellular fractionation studies by western blotting and IHC analysis in TH mice. Altogether these studies report a rise in cardiac biomarkers such as CTnI and hFABP, that correlate with gross changes in mitochondrial ultra structures there by triggering the translocation and activation of death inducing factors such as AIF in these TH mice models. Mitochondrial damage has been noted previously in trauma but as a result of tissue injury. Studies have reported the inflammatory response induced by mitochondrial DAMPs (164). I propose that the cell injury is induced by mitochondrial dysfunction in itself.

Chapter 6

Discussion

6.1 Summary of Findings

The prospective cohort study of 290 patients demonstrated the a 13% prevalence of TISCI as defined by clinical events, ECGs and rise in h-FABP. This correlated with previous retrospective studies (32). Unlike the temporal trend of h-FABP in pathologies of myocardial stress such as PE where it rises and falls within 24 hours, in TISCI, it remains raised throughout the first 72 hours (72). This raises the possibility that TISCI occurs over days and the ongoing ischaemia does not resolve with resuscitation. Patients who had higher levels of h-FABP on admission had worse clinical outcomes including increased mortality, longer length of hospital stay, prolonged critical admissions and increased inotropic requirement. In an age of increasing need for resource management particularly within the NHS, this model for identifying patients with TISCI could prove to be an important tool in prediction of patient placement during admission.

Furthermore, this study highlights an opportunity to identify patients with subclinical coronary artery disease. In patients who develop transient ischaemic changes with pre-existing cardiac risk factors, cardiology follow up should be considered on discharge for further investigation and management.

Interestingly, hypertension was revealed as a significant contributor to TISCI, the reasons for this may be multifactorial. Hypertension not only induces endothelial damage but also leads to changes to the myocardium and predisposes to the development of arrhythmias (144). It may also be that hypertensive patients

accustomed to higher coronary perfusion pressures, even a small degree of hypotension leads to a greater degree of cardiac ischaemia (165).

This study further demonstrated the role of catecholamines in TISCI. Serum adrenaline and noradrenaline were higher in patients who developed ACE and predicted inotropic requirement. Furthermore, adrenaline and noradrenaline levels had an incremental effect on h-FABP levels, provoking thoughts of effect. However, on multivariate analysis, these were not calculated to be independent factors, potentially due to their co-linearity with degree of shock and injury severity.

Murine models enabled further examination and validation of increase in cardiac injury associated biomarkers. The increase in serum h-FABP in these models is associated with reduction in its tissue concentration. Furthermore tissue expression of troponin-I was also reduced. The most impressive finding was the degree of changes in ultra fine structures of sub cellular components such as myofibril organisation and mitochondrial fission as analysed by electron microscopy in the fixed sections of cardiac tissue. There is definite evidence of ischaemia displayed as widespread oedema, organelle swelling and mitochondrial changes. This alongside the translocation of the death-related mitochondrial protein AIF into the cytosol highlights the role of the mitochondria in TISCI.

6.2 Strengths of this Work

The Centre for Trauma Sciences at Blizard Institute Queen Mary University of London provides an excellent structure for translational research through collaborative efforts of clinicians from pre-hospital to rehabilitation and the research fellows. The assiduous data collection by research fellows who are clinicians with a dedicated interest in trauma sciences attests the integrity of the data. This facilitated with ease the prospective recruitment and substantial data collection for the clinical study. Due to the prospective design, the contributions of past medical history as well as medications were explored, in addition the effect of ACE on inotropic support.

Trauma haemorrhage is a complex entity with bleeding and tissue injury leading to a plethora of noxious events including but not limited to hypoperfusion, bleeding diasthesis, hyperadrenergic stimuli which all mediates cellular responses leading to inflammation, multi-organ failure and death. To replicate this, is not a slight task and I was fortunate enough to be in a group with those extremely talented in animal modeling. Not only did they produce trauma haemorrhage models but they also confirmed the presence of TISCI in the models. Ease of access to the hearts from the TISCI models immensely strengthened the work produced in this thesis by facilitating the exploration in to the mechanisms underpinning cardiac injury. This led to the confirmation of cell death in the myocardium, validating cardiac injury as suggested by the increased blood levels of cardiac biomarkers and the novel identification of mitochondrial abnormalities on histology and the subsequent release of mitochondrial death protein AIF.

6.3 Limitations of This Work

An important aspect of TACID was to obtain functional cardiac measures in trauma patients. Unpredicted resource limitations and coinciding restructuring of services at our hospital lead to poor compliance with echocardiogram examinations in this cohort. Despite plans to obtain echocardiograms for all recruited patients, only 15% of patients had one completed and more importantly only 44% of the patients who developed ACE had echocardiograms. Surprisingly, despite higher rates of initiation of inotropes in the ACE group, analysis of echocardiogram results did not reveal any difference in cardiac function in the TH patients. Due to the variability in time of examination and patient selection, I am reluctant to put this forward as an absolute finding. Furthermore, the echocardiography parameters that were reported within the completed studies are all sensitive to changes in volume, although they were completed following the initial resuscitative period where one may assume the patients are euvolaemic, it will still be affected by changes in volume distribution as seen in SIRS following trauma. It would have been pertinent to examine more robust parameters such as tissue doppler index and global longitudinal strain. Further functional data could have also been collected using dynamic cardiac monitoring. For the duration of the TACID study, LiDCO availability was limited. Nonetheless, use of LiDCO brings its own limitations, with difficulty in calibration and need for expertise in utilisation, and therefore may not have been particularly helpful in this study. Furthermore, not all patients got ECGs at all time points, however they were still pragmatically included in the analysis.

Financial restraints necessitated omission of troponin measurement at 24 and 72 hours. The troponin examination would have shed light on the temporal trend of TISCI in addition to h-FABP. Furthermore, to allow for seamless translation of this work into clinical practice, measurements should have been made using an ultra-sensitive troponin assay. However, on a small scale without an established clinical indication, it was not possible to collaborate with the hospital lab. These assays would also have to been performed on dedicated machines which require outsourcing to the manufacturers and provide further budgetary demands. A weakness of the catecholamine ELISA is that exogenous and endogenous catecholamines cannot be differentiated, however, to answer the question whether catecholamine exposure is associated with poorer outcomes, I felt an understanding of the temporal trend of catecholamine levels at 24 and 72 hours would be useful. I did not anticipate the magnitude of the contribution of exogenous catecholamines and potentially an insignificant contribution of endogenous catecholamines. Especially, as often catecholamine use beyond the initial resuscitative stage can be necessary and unavoidable, this is an unlikely management strategy that would be amenable to change to potentially improve outcomes. On hindsight, resources may have been better spent focusing on troponin at 24 and 72 hours.

Despite the sophistication of the TISCI murine models, direct translation of animal data to humans should be done with caution. Small animal models such as mice and rats are frequently used due to their ease of handling and low costs. Despite sharing 80% of their genes with humans, they are still relatively genetically distant. Gene

expression studies in animal trauma models have shown low correlation with human genomic change (166). This can be overcome with correlation studies of genomic changes induced by trauma in mice and humans with subsequent human cell studies to aid translation. The rodents' dissimilarity to humans make them insufficient as a stand alone model for development of novel therapeutic substances but one may argue sufficient for repurposing well established treatments.

Although in combination, IHC and western blotting are useful for understanding pathologies, there are still many limitations. Western blotting provides an overarching measure of the protein expression whereas IHC enables study of protein distribution. In the data presented, the changes in expression did not correlate between the two methods, for example the total AIF expression. Despite a large number of repeated measures, IHC still only allows investigation of a small region of the whole organ whereas western blot accounts for the organ as a whole, so the estimation from western blotting is more likely to be representative of the global protein expression.

Furthermore, neither of these are essentially quantitative methods. Technological innovations have enabled conversion of these techniques in to quantitative methods and now, this is well-established practice. However, it is still important to correlate the numbers with blots and sections. Sophisticated software for calculating immunofluorescence and localisation index has made data easier to manage, interpret and present. However, a lot of information can be lost or

misinterpreted. There is still there no better substitute for careful visual interrogation.

Although, the involvement of AIF in cardiac dysfunction and TH is novel and exciting, this needs further validation with gene expression analysis.

6.4 Future Work

The evidence from the clinical studies highlights the prevalence of TISCI in our trauma population. Especially in light of the evidence regarding the prevalence of cardiovascular disease in out of hospital deaths, ACE in-hospital should instigate further investigation. Surprisingly, only 44% of those that developed an ACE went on to have an echocardiogram, this was mainly due to limitations of expertise in the technique. Echocardiograms are now much more utilised due to increase in multi-disciplinary training as well as the recognition of their utility in prognostication in many disease processes. Future work is needed to increase clinical awareness of TISCI as a prognostic marker in trauma patients to instigate further investigation through an echocardiogram and arrange appropriate follow up either in secondary or primary care.

The recognition of mitochondrial involvement and translocation of AIF has given our research a new focus. The release of AIF is facilitated by the Bcl-2 family proteins as well as the permeability transition pore of mitochondria (167). Recent human genomic studies of critically injured patients revealed the upregulation of mitochondrial stress and respiration genes overall and further more, upregulation

of cell death related genes in multi-organ failure (168). The changes seen in the murine myocardium may potentially be reflective changes and intrinsic to multiorgan failure. Follow-up experiments replicated in stored lung and kidney tissues from the same models could aid further understanding. Multiple pre-clinical studies have highlighted melatonin as a potential candidate for alleviating mitochondrial swelling and permeability transition pore opening in ischaemia reperfusion, muscle injury and neurodegenerative diseases (169-171). Clinical studies showing reduction in intra-operative bleeding, post operative cardiac complications and multi organ dysfunction in sepsis in children and burns (170,172). Melatonin is known to be safe in high doses and it is widely used in critical care to reduce delirium and aid extubation, therefore, a relatively safe therapeutic agent to explore(170). Further investigation in to therapeutic doses and effect on organ dysfunction and apoptosis in murine studies may be beneficial. Additionally, studies on the protein structure and interactions of AIF have suggested both hsp70 and XIAP as potential targets for mitigating apoptosis. However, therapeutic molecules to potentiate their anti-apoptotic properties are still being developed (167). Therefore characterisation of various proteins that regulate apoptotic signaling cascades could insight clues to disease mechanisms in these TH mice models. Moreover, ultra structural changes in mitochondria and nuclear chromatin might possibly indicate changes in genome architecture and assembly. This could lead to increase in DNA damage response and changes in transcriptional state of cells in these mice. Further exploration to understand the basic biology underlying these mice models could provide insight into disease mechanisms and therefore open

new avenues for targeted therapy and improved management of care in trauma patients

6.4 Conclusions

The studies in this thesis have demonstrated the presence of TISCI in the trauma population associated with hypertension and raised noradrenaline and adrenaline levels on admission which predicted inotropic use, length of ICU stay and mortality. Furthermore, in the animal models of TISCI revealed significant ischaemic changes on histology with signs of mitochondrial damage, nuclear condensation and translocation of mitochondrial death protein AIF to its active site. This poses a new question for further research and a potential target for treatment.

References

1. Norton R, Kobusingye O. Injuries. N Engl J Med. 2013 May 2;368(18):1723–30.

2. National Audit Office. Major trauma care in England. 2010 Feb 3;:1–41.

3. WHO. Health in 2015: From MDG to SDG. Geneva; 2015 Dec 1;:1–18.

4. WHO. Global Health Estimates 2015: Deaths by Cause, Age, Sex, by Country and by Region. Geneva; 2016.

5. Soberg HL, Bautz-Holter E, Roise O, Finset A. Long-Term Multidimensional Functional Consequences of Severe Multiple Injuries Two Years After Trauma: A Prospective Longitudinal Cohort Study. The Journal of Trauma: Injury, Infection, and Critical Care. 2007 Feb;62(2):461–70.

6. PARKER MM. Profound but Reversible Myocardial Depression in Patients with Septic Shock. Ann Intern Med. 1984 Apr 1;100(4):483.

7. Evans JA, van Wessem KJP, McDougall D, Lee KA, Lyons T, Balogh ZJ. Epidemiology of Traumatic Deaths: Comprehensive Population-Based Assessment. World J Surg. 2009 Oct 31;34(1):158–63.

8. Rudiger A, Singer M. Mechanisms of sepsis-induced cardiac dysfunction. Critical Care Medicine. 2007 Jun;35(6):1599–608.

9. Cole E, Lecky F, West A, Smith N, Brohi K, Davenport R. The Impact of a Pan-regional Inclusive Trauma System on Quality of Care. Annals of Surgery. 2016 Jul;264(1):188–94.

10. Sauaia A, Moore EE, Johnson JL, Chin TL, Banerjee A, Sperry JL, et al. Temporal trends of postinjury multiple-organ failure. Journal of Trauma and Acute Care Surgery. 2014 Mar;76(3):582–93.

11. Witthaut R, Busch C, Fraunberger P, Walli A, Seidel D, Pilz GN, et al. Plasma atrial natriuretic peptide and brain natriuretic peptide are increased in septic shock: impact of interleukin-6 and sepsis-associated left ventricular dysfunction. Intensive Care Med. 2003 Oct 1;29(10):1696–702.

12. Rudiger A, Gasser S, Fischler M, Hornemann T, Eckardstein von A, Maggiorini M. Comparable increase of B-type natriuretic peptide and amino-terminal pro-B-type natriuretic peptide levels in patients with severe sepsis, septic shock, and acute heart failure*. Critical Care Medicine. 2006 Aug;34(8):2140–4.

13. Fröhlich M, Lefering R, Probst C, Paffrath T, Schneider MM, Maegele M, et al. Epidemiology and risk factors of multiple-organ failure after multiple trauma. Journal of Trauma and Acute Care Surgery. 2014 Apr;76(4):921–8.

14. Probst C, Zelle BA, Sittaro NA, Lohse R, Krettek C, Pape HC. Late Death After Multiple Severe Trauma: When Does It Occur and What Are the Causes? The Journal of Trauma: Injury, Infection, and Critical Care. 2009 Apr;66(4):1212–7.

15. Remmers DE, Cioffi WG, Bland KI, Wang P, Angele MK, Chaudry IH. Testosterone: the crucial hormone responsible for depressing myocardial function in males after trauma-hemorrhage. Annals of Surgery. 1998 Jun;227(6):790–9.

16. Mizushima Y, Wang P, Jarrar D, Cioffi WG, Bland KI, Chaudry IH. Preinduction of heat shock proteins protects cardiac and hepatic functions following trauma and hemorrhage. Am J

Physiol Regul Integr Comp Physiol. 2000 Feb;278(2):R352–9.

17. Mizushima Y, Wang P, Jarrar D, Cioffi WG, Bland KI, Chaudry IH. Estradiol administration after trauma-hemorrhage improves cardiovascular and hepatocellular functions in male animals. Annals of Surgery. 2000 Nov;232(5):673–9.

18. Yang S. Mechanism of cardiac depression after trauma-hemorrhage: increased cardiomyocyte IL-6 and effect of sex steroids on IL-6 regulation and cardiac function. AJP: Heart and Circulatory Physiology. 2004 Jul 8;287(5):H2183–91.

19. Szalay L. Mechanism of salutary effects of estradiol on organ function after trauma-hemorrhage: upregulation of heme oxygenase. AJP: Heart and Circulatory Physiology. 2005 Jul 1;289(1):H92–8.

20. Yu H-P, Yang S, Choudhry MA, Hsieh Y-C, Bland KI, Chaudry IH. Mechanism responsible for the salutary effects of flutamide on cardiac performance after trauma-hemorrhagic shock: Upregulation of cardiomyocyte estrogen receptors. Surgery. 2005 Jul;138(1):85–92.

21. Yang S, Hu S, Hsieh Y-C, Choudhry MA, Rue LW III, Bland KI, et al. Mechanism of IL-6-mediated cardiac dysfunction following trauma-hemorrhage. Journal of Molecular and Cellular Cardiology. 2006 Apr;40(4):570–9.

22. Hsu JT, Kan WH, Hsieh CH, Choudhry MA, Schwacha MG, Bland KI, et al. Mechanism of estrogen-mediated intestinal protection following trauma-hemorrhage: p38 MAPK-dependent upregulation of HO-1. AJP: Regulatory, Integrative and Comparative Physiology. 2008 Apr 2;294(6):R1825–31.

23. Kan WH, Hsu JT, Ba ZF, Schwacha MG, Chen J, Choudhry MA, et al. p38 MAPK-dependent eNOS upregulation is critical for 17 -estradiol-mediated cardioprotection following trauma-hemorrhage. AJP: Heart and Circulatory Physiology. 2008 Feb 29;294(6):H2627–36.

24. Yu H-P, Chaudry IH. THE ROLE OF ESTROGEN AND RECEPTOR AGONISTS IN MAINTAINING ORGAN FUNCTION AFTER TRAUMA-HEMORRHAGE. Shock. 2009 Mar;31(3):227–37.

25. Hsu J-T, Kan W-H, Hsieh C-H, Choudhry MA, Bland KI, Chaudry IH. Mechanism of salutary effects of estrogen on cardiac function following trauma-hemorrhage: Akt-dependent HO-1 up-regulation*. Critical Care Medicine. 2009 Aug;37(8):2338–44.

26. Hadjizacharia P, O'Keeffe T, Brown CVR, Inaba K, Salim A, Chan LS, et al. Incidence, risk factors, and outcomes for atrial arrhythmias in trauma patients. Am Surg. 2011 May;77(5):634–9.

27. Kuebler JF, Jarrar D, Bland KI, Rue L III,, Wang P, Chaudry IH. Progesterone administration after trauma and hemorrhagic shock improves cardiovascular responses. Critical Care Medicine. 2003 Jun;31(6):1786–93.

28. Yang S, Hu S, Choudhry MA, Rue LW III, Bland KI, Chaudry IH. Anti-rat soluble IL-6 receptor antibody down-regulates cardiac IL-6 and improves cardiac function following trauma–hemorrhage. Journal of Molecular and Cellular Cardiology. 2007 Mar;42(3):620–30.

29. Ismailov RM, Ness RB, Weiss HB, Lawrence BA, Miller TR. Trauma associated with acute myocardial infarction in a multi-state hospitalized population. International Journal of Cardiology. 2005 Nov;105(2):141–6.

30. Seguin P, Signouret T, Laviolle B, Branger B, Mallédant Y. Incidence and risk factors of atrial fibrillation in a surgical intensive care unit*. Critical Care Medicine. 2004 Mar;32(3):722–6.

31. Seguin P, Laviolle B, Maurice A, Leclercq C, Mallédant Y. Atrial fibrillation in trauma patients requiring intensive care. Intensive Care Med. 2006 Jan 27;32(3):398–404.

32. De'Ath HD, Rourke C, Davenport R, Manson J, Renfrew I, Uppal R, et al. Clinical and biomarker profile of trauma-induced secondary cardiac injury. Br J Surg. 2012 Mar 21;99(6):789–97.

33. De'Ath HD, Manson J, Davenport R, Glasgow S, Renfrew I, Davies LC, et al. Trauma-Induced Secondary Cardiac Injury Is Associated With Hyperacute Elevations in Inflammatory Cytokines. Shock. 2013 May;39(5):415–20.

34. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third Universal Definition of Myocardial Infarction. Circulation. 2012 Oct 15;126(16):2020–35.

35. Sarko J, Pollack CV. Cardiac troponins1 1Clinical Laboratory in Emergency Medicine is coordinated by Jonathan Olshaker, MD, of the University of Maryland Medical Center, Baltimore, Maryland. The Journal of Emergency Medicine. 2002;23(1):57–65.

36. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. European Heart Journal. 2012 Sep 14;33(18):2252–7.

37. Agewall S, Giannitsis E, Jernberg T, Katus H. Troponin elevation in coronary vs. non-coronary disease. European Heart Journal. 2011 Feb 14;32(4):404–11.

38. Kostrubiec M. Biomarker-based risk assessment model in acute pulmonary embolism. European Heart Journal. 2005 Sep 1;26(20):2166–72.

39. Maziere F, Birolleau S, Medimagh S, Arthaud M, Bennaceur M, Riou B, et al. Comparison of troponin I and N-terminal-pro B-type natriuretic peptide for risk stratification in patients with pulmonary embolism. European Journal of Emergency Medicine. 2007;14(4).

40. Ammann P, Fehr T, Minder E, Günter C, Bertel O. Elevation of troponin I in sepsis and septic shock. Intensive Care Med. 2001 May 16;27(6):965–9.

41. Arlati S, Brenna S, Prencipe L, Marocchi A, Casella GP, Lanzani M, et al. Myocardial necrosis in ICU patients with acute non-cardiac disease: a prospective study. Intensive Care Med. 2000 Jan;26(1):31–7.

42. Mehta NJ, Khan IA, Gupta V, Jani K, Gowda RM, Smith PR. Cardiac troponin I predicts myocardial dysfunction and adverse outcome in septic shock. International Journal of Cardiology. 2004;95(1):13–7.

43. Varpula M, Pulkki K, Karlsson S, Ruokonen E, Pettilä V. Predictive value of N-terminal pro–brain natriuretic peptide in severe sepsis and septic shock*. Critical Care Medicine. 2007 May;35(5):1277–83.

44. Bessière F, Khenifer S, Dubourg J, Durieu I, Lega J-C. Prognostic value of troponins in sepsis: a meta-analysis. Intensive Care Med. 2013 Apr 18;39(7):1181–9.

45. Guest TM. Myocardial Injury in Critically Ill Patients. JAMA. 1995 Jun 28;273(24):1945.

46. Quenot J-P, Le Teuff G, Quantin C, Doise J-M, Abrahamowicz M, Masson D, et al. Myocardial Injury in Critically Ill Patients: Relation to Increased Cardiac Troponin I and Hospital Mortality. Chest. 2005;128(4):2758–64.

47. de Lemos JA, Morrow DA, Bentley JH, Omland T, Sabatine MS, McCabe CH, et al. The

Prognostic Value of B-Type Natriuretic Peptide in Patients with Acute Coronary Syndromes. N Engl J Med. 2001 Oct 4;345(14):1014–21.

48. McDonagh TA, Holmer S, Raymond I, Luchner A, Hildebrant P, Dargie HJ. NT-proBNP and the diagnosis of heart failure: a pooled analysis of three European epidemiological studies. European Journal of Heart Failure. 2004 Mar 1;6(3):269–73.

49. Richards AM. B-Type Natriuretic Peptides and Ejection Fraction for Prognosis After Myocardial Infarction. Circulation. 2003 Jun 10;107(22):2786–92.

50. Talwar S. Profile of plasma N-terminal proBNP following acute myocardial infarction. Correlation with left ventricular systolic dysfunction. European Heart Journal. 2000 Sep 15;21(18):1514–21.

51. Karthikeyan G, Moncur RA, Levine O, Heels-Ansdell D, Chan MTV, Alonso-Coello P, et al. Is a Pre-Operative Brain Natriuretic Peptideor N-Terminal Pro–B-Type Natriuretic Peptide Measurement an Independent Predictor of Adverse Cardiovascular Outcomes Within30 Days of Noncardiac Surgery? JAC. Elsevier Inc; 2009 Oct 20;54(17):1599–606.

52. Peden M. World Health Organization dedicates World Health Day to road safety. Injury Prevention. 2004 Apr 1;10(2):67–7.

53. MD PRL, PhD MN, MPH KF, PhD SL, MD PKS, MD PVA, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet. Elsevier Ltd; 2012 Dec 15;380(9859):2095–128.

54. Pruszczyk P, Kostrubiec M, Bochowicz A, Styczyński G, Szulc M, Kurzyna M, et al. N-terminal pro-brain natriuretic peptide in patients with acute pulmonary embolism. Eur Respir J. 2003 Oct 1;22(4):649–53.

55. Norton R, Kobusingye O. Injuries. N Engl J Med. 2013 May 2;368(18):1723–30.

56. Kostrubiec M, Pruszczyk P, Kaczyñska A, Kucher N. Persistent NT-proBNP elevation in acute pulmonary embolism predicts early death. Clinica Chimica Acta. 2007;382(1):124–8.

57. Haagsma JA, Graetz N, Bolliger I, Naghavi M, Higashi H, Mullany EC, et al. The global burden of injury: incidence, mortality, disability-adjusted life years and time trends from the Global Burden of Disease study 2013. Injury Prevention. 2015 Dec 3;:injuryprev–2015–041616.

58. Lega J-C, Lacasse Y, Lakhal L, Provencher S. Natriuretic peptides and troponins in pulmonary embolism: a meta-analysis. Thorax. 2009 Sep 28;64(10):869–75.

59. Kostrubiec M. Biomarker-based risk assessment model in acute pulmonary embolism. European Heart Journal. 2005 Sep 1;26(20):2166–72.

60. McLean AS, Huang SJ, Hyams S, Poh G, Nalos M, Pandit R, et al. Prognostic values of B-type natriuretic peptide in severe sepsis and septic shock*. Critical Care Medicine. 2007 Apr;35(4):1019–26.

61. Ueda S, Nishio K, Akai Y, Fukushima H, Ueyama T, Kawai Y, et al. PROGNOSTIC VALUE OF INCREASED PLASMA LEVELS OF BRAIN NATRIURETIC PEPTIDE IN PATIENTS WITH SEPTIC SHOCK. Shock. 2006 Aug;26(2):134–9.

62. Wang F, Wu Y, Tang L, Zhu W, Chen F, Xu T, et al. Brain natriuretic peptide for prediction ofmortality in patients with sepsis: a systematicreview and meta-analysis. Crit Care.

BioMed Central Ltd; 2013 May 6;16(3):R74.

63. Tung RH, Garcia C, Morss AM, Pino RM, Fifer MA, Thompson BT, et al. Utility of B-type natriuretic peptide for the evaluation of intensive care unit shock*. Critical Care Medicine. 2004 Aug;32(8):1643–7.

64. Meyer B, Huelsmann M, Wexberg P, Karth GD, Berger R, Moertl D, et al. N-terminal pro-B-type natriuretic peptide is an independent predictor of outcome in an unselected cohort of critically ill patients*. Critical Care Medicine. 2007 Oct;35(10):2268–73.

65. Lippi G, Mattiuzzi C, Cervellin G. Clinical Biochemistry. Clinical Biochemistry. The Canadian Society of Clinical Chemists; 2013 Jan 1;46(1-2):26–30.

66. Pelsers MMAL. Brain- and Heart-Type Fatty Acid-Binding Proteins in the Brain: Tissue Distribution and Clinical Utility. Clinical Chemistry. 2004 Sep 1;50(9):1568–75.

67. Kilcullen N, Viswanathan K, Das R, Morrell C, Farrin A, Barth JH, et al. Heart-Type Fatty Acid-Binding Protein Predicts Long-Term Mortality After Acute Coronary Syndrome and Identifies High-Risk Patients Across the Range of Troponin Values. Journal of the American College of Cardiology. 2007 Nov;50(21):2061–7.

68. Ishii J. Prognostic Value of Serum Concentration of Heart-Type Fatty Acid-Binding Protein Relative to Cardiac Troponin T on Admission in the Early Hours of Acute Coronary Syndrome. Clinical Chemistry. 2005 Aug 1;51(8):1397–404.

69. O'Donoghue M. Prognostic Utility of Heart-Type Fatty Acid Binding Protein in Patients With Acute Coronary Syndromes. Circulation. 2006 Aug 8;114(6):550–7.

70. Wunderlich C, Langer M, Richter U, Tausche AK, Sveric K, Boscheri A, et al. Combined value of heart-type fatty acid-binding protein and myocardial creatine kinase in risk stratification of normotensive patients with pulmonary embolism. European Heart Journal. 2013 Sep 3;34(suppl 1):761–1.

71. Lankeit M, Dellas C, Benz V, Hasenfuß G, Konstantinides S. Thrombosis Research. Thrombosis Research. Elsevier B.V; 2013 Nov 1;132(5):543–7.

72. Dellas C, Puls M, Lankeit M, Schäfer K, Cuny M, Berner M, et al. Elevated Heart-Type Fatty Acid-Binding Protein Levels on Admission Predict an Adverse Outcome in Normotensive Patients With Acute Pulmonary Embolism. JAC. Elsevier Inc; 2010 May 11;55(19):2150–7.

73. VUILLEUMIER N, LE GAL G, VERSCHUREN F, PERRIER A, BOUNAMEAUX H, TURCK N, et al. Cardiac biomarkers for risk stratification in non-massive pulmonary embolism: a multicenter prospective study. Journal of Thrombosis and Haemostasis. 2009 Mar;7(3):391–8.

74. Bajaj A, Rathor P, Kabak B, Shetty A, Sehgal V, Hosur S. Prognostic Value of Heart-Type Fatty Acid Binding Protein in Sepsis: A Meta-Analysis. In: American Thoracic Society International Conference Abstracts. American Thoracic Society; 2015. pp. A2569–9.

75. Kaczyñska A, Pelsers MMAL, Bochowicz A, Kostrubiec M, Glatz JFC, Pruszczyk P. Plasma heart-type fatty acid binding protein is superior to troponin and myoglobin for rapid risk stratification in acute pulmonary embolism. Clinica Chimica Acta. 2006 Sep;371(1-2):117–23.

76. Liu M, Yuan X, Qiu X, Shan X, Lin D, Zhu L. Thrombosis Research. Thrombosis Research. Elsevier Ltd; 2015 Jan 1;135(1):20–5.

77. Gul EE, Can I, Kayrak M, Abdulhalikov T, Erdogan HI, Altunbas G, et al. Thrombolysis in patients with pulmonary embolism and elevated heart-type fatty acid-binding protein levels. J Thromb Thrombolysis. 2013 Nov 22;37(4):483–9.

78. Chen Y-X, Li C-S. Journal of Critical Care. Journal of Critical Care. Elsevier Inc; 2014 Aug 1;29(4):512–6.

79. Zhang Z-C, Dai H-W, Yu Y-HY, Yang J-DY, Hu C-B. Usefulness of heart-type fatty acid. Journal of Critical Care. Elsevier Inc; 2012 Aug 1;27(4):415.e13–8.

80. Jo YH, Kim KK, Lee JH, Rhee JE, Lee JH, Kang KW, et al. Heart-type fatty acid. American Journal of Emergency Medicine. Elsevier Inc; 2012 Nov 1;30(9):1749–55.

81. Clancy K, Velopulos C, Bilaniuk JW, Collier B, Crowley W, Kurek S, et al. Screening for blunt cardiac injury. Journal of Trauma and Acute Care Surgery. 2012 Nov;73:S301–6.

82. Salim A, Velmahos GC, Jindal A, Chan L, Vassiliu P, Belzberg H, et al. Clinically Significant Blunt Cardiac Trauma: Role of Serum Troponin Levels Combined with Electrocardiographic Findings. Journal of Trauma and Acute Care Surgery. 2001;50(2).

83. Rajan GP, Zellweger R. Cardiac Troponin I as a Predictor of Arrhythmia and Ventricular Dysfunction in Trauma Patients With Myocardial Contusion. Journal of Trauma and Acute Care Surgery. 2004;57(4).

84. Lagi A, Meucci E, Cencetti S. Outcome of patients with elevated cardiac troponin I level after mild trauma. American Journal of Emergency Medicine. 2008;26(2):248.e3–248.e5.

85. Monaghan SF, Adams CA Jr., Stephen AH, Connolly MD, Gregg SC, Machan JT, et al. Infections after trauma are associated with subsequent cardiac injury. Journal of Trauma and Acute Care Surgery. 2012 Nov;73(5):1079–85.

86. Sangha GS, Pepelassis D, Buffo-Sequeira I, Seabrook JA, Fraser DD. Serum troponin-I as an indicator of clinically significant myocardial injury in paediatric trauma patients. Injury. 2012;43(12):2046–50.

87. Kirchhoff C, Leidel BA, Kirchhoff S, Braunstein V, Bogner V, Kreimeier U, et al. Analysis of N-terminal pro-B-type natriuretic peptide and cardiac index in multiple injured patients: a prospective cohort study. Crit Care. 2008;12(5):R118.

88. Friese RS, Dineen S, Jennings A, Pruitt J, McBride D, Shafi S, et al. Serum B-Type Natriuretic Peptide: a Marker of Fluid Resuscitation After Injury? The Journal of Trauma: Injury, Infection, and Critical Care. 2007 Jun;62(6):1346–51.

89. Bouras P, Rogdakis A, El-Fellach N, Karampas B, Liakos G, Mavrodakou B, et al. Plasma B-type natriuretic peptide (BNP) variation in multi-trauma patients. Hellenic Journal of Surgery. 2011;83(4):202.

90. Vander Werf BD, Watt J, Joseph B, Wynne J, Kulvatunyou N, O'Keeffe T, et al. Can plasma B-type natriuretic peptide levels predict need for mechanical ventilation after injury? AJS. 2010;200(6):845–50.

91. Stewart D, Waxman K, Brown CA, Schuster R, Schuster L, Hvingelby EM, et al. B-Type Natriuretic Peptide Levels May Be Elevated in the Critically Injured Trauma Patient Without Congestive Heart Failure. The Journal of Trauma: Injury, Infection, and Critical Care. 2007 Oct;63(4):747–50.

92. Akpinar G, Duman A, Gulen B, Kapci M, Altinbilek E, Ikizceli I. Role of H-FABP values in

determining the etiologic factors of the cardiac injuries. Pan Afr Med J. 2017;26.

93. Walder B, Robin X, Rebetez MML, Copin J-C, Gasche Y, Sanchez J-C, et al. The Prognostic Significance of the Serum Biomarker Heart-Fatty Acidic Binding Protein in Comparison with S100b in Severe Traumatic Brain Injury. Journal of Neurotrauma. 2013 Oct;30(19):1631–7.

94. Celes MRN, Prado CM, Rossi MA. Sepsis: Going to the Heart of the Matter. Pathobiology. 2012 Sep 10;80(2):70–86.

95. Salem R, Vallée F, Depret F, Callebert J, Saint Maurice JP, Marty P, et al. Subarachnoid hemorrhage induces an early and reversible cardiac injury associated with catecholamine release: one-week follow-up study. 2014 Oct 30;18(5):1–10.

96. Arbabi S, Ahrns KS, Wahl WL, Hemmila MR, Wang SC, Brandt M-M, et al. Beta-Blocker Use Is Associated with Improved Outcomes in Adult Burn Patients. The Journal of Trauma: Injury, Infection, and Critical Care. 2004 Feb;56(2):265–71.

97. Cotton BA, Snodgrass KB, Fleming SB, Carpenter RO, Kemp CD, Arbogast PG, et al. Beta-Blocker Exposure is Associated With Improved Survival After Severe Traumatic Brain Injury. The Journal of Trauma: Injury, Infection, and Critical Care. 2007 Jan;62(1):26–35.

98. Christensen S, Johansen MB, Tønnesen E, Larsson A, Pedersen L, Lemeshow S, et al. Preadmission beta-blocker use and 30-daymortality among patients in intensive care:a cohort study. Crit Care. BioMed Central Ltd; 2011 Mar 7;15(2):R87.

99. Neideen T, Lam M, Brasel KJ. Preinjury Beta Blockers are Associated With Increased Mortality in Geriatric Trauma Patients. The Journal of Trauma: Injury, Infection, and Critical Care. 2008 Nov;65(5):1016–20.

100. Cebelin MS, Hirsch CS. Human stress cardiomyopathy: Myocardial lesions in victims of homicidal assaults without internal injuries. Human Pathology. 1980;11(2):123–32.

101. Yoshida K, Ogura Y, Wakasugi C. Myocardial lesions induced after trauma and treatment. Forensic Sci Int. 1992 May;54(2):181–9.

102. Gawande NB, Tumram NK, Dongre AP. Cardiac Changes in Hospitalized Patients of Trauma. Shock. 2014 May;:1.

103. Li S, Tao L, Jiao X, Liu H, Cao Y, Lopez B, et al. TNFα-initiated oxidative/nitrative stress mediates cardiomyocyte apoptosis in traumatic animals. Apoptosis. 2007 Aug 14;12(10):1795–802.

104. Yang L, Lin J, Zhao X, Yao L, Ding Q, Chai W. Exogenous hydrogen sulfide restores cardiac function after trauma-hemorrhagic shock by inhibiting mitochondrial apoptosis. Int J Clin Exp Med. 2016;9(3):5563–73.

105. Hsieh Y-C, Yu H-P, Suzuki T, Choudhry MA, Schwacha MG, Bland KI, et al. Upregulation of mitochondrial respiratory complex IV by estrogen receptor-β is critical for inhibiting mitochondrial apoptotic signaling and restoring cardiac functions following trauma–hemorrhage. Journal of Molecular and Cellular Cardiology. 2006 Sep;41(3):511–21.

106. Orogo AM, Gustafsson ÅB. Cell death in the myocardium: My heart won't go on. IUBMB Life. 2013 Jul 3;65(8):651–6.

107. Leist M, Jäättelä M. Four deaths and a funeral: from caspases to alternative mechanisms. Nature Publishing Group. 2001 Aug;2(8):589–98.

108. Hostmann A, Jasse K, Schulze-Tanzil G, Robinson Y, Oberholzer A, Ertel W, et al. Biphasic onset of splenic apoptosis following hemorrhagic shock: critical implications for Bax, Bcl-2 and Mcl-1 proteins. Crit Care. 2008;12(1):R8.

109. Grigoryev EG, Radivilko AR, Kiseleva AK, Churliaev YC, Vavin GV. Diagnostic and prognostic significance of serum apoptosis markers in the patients with severe trauma injury. Crit Care. 2010;14(1):P286.

110. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Brenner C, Larochette N, et al. Mitochondrial Release of Caspase-2 and -9 during the Apoptotic Process. J Exp Med. The Rockefeller University Press; 1999 Jan 18;189(2):381–94.

111. Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides. Elsevier Inc; 2015 Oct 1;72(C):4–15.

112. Ramos-Vara JA, Kiupel M, Baszler T, Bliven L, Brodersen B, Chelack B, et al. Suggested Guidelines for Immunohistochemical Techniques in Veterinary Diagnostic Laboratories. J VET Diagn Invest. 4 ed. 2008 Jul;20(4):393–413.

113. Vantomme B, Donck J, van Hooland S, Wauters A, De Clippele M, Neirynck V, et al. Diagnosis of pheochromocytoma in a hemodialysis patient through measurement of plasma catecholamines. Hemodialysis International. 2016 Jan 7;20(3):E6–E9.

114. Sabatini DD. CYTOCHEMISTRY AND ELECTRON MICROSCOPY: The Preservation of Cellular Ultrastructure and Enzymatic Activity by Aldehyde Fixation. The Journal of Cell Biology. 1963 Apr 1;17(1):19–58.

115. Reynolds ES. THE USE OF LEAD CITRATE AT HIGH pH AS AN ELECTRON-OPAQUE STAIN IN ELECTRON MICROSCOPY. The Journal of Cell Biology. The Rockefeller University Press; 1963 Apr 1;17(1):208–12.

116. Velmahos GC, Demetriades D, Shoemaker WC, Chan LS, Tatevossian R, Wo CC, et al. Endpoints of resuscitation of critically injured patients: normal or supranormal? A prospective randomized trial. Annals of Surgery. 2000 Sep;232(3):409–18.

117. Blow O, Magliore L, Claridge JA, Butler K, Young JS. The Golden Hour and the Silver Day: Detection and Correction of Occult Hypoperfusion within 24 Hours Improves Outcome from Major Trauma. Journal of Trauma and Acute Care Surgery. 1999;47(5).

118. De'Ath HD, Rourke C, Davenport R, Manson J, Renfrew I, Uppal R, et al. Clinical and biomarker profile of trauma-induced secondary cardiac injury. Br J Surg. 2012 Mar 21;99(6):789–97.

119. Bruins Slot MHE, Reitsma JB, Rutten FH, Hoes AW, van der Heijden GJMG. Heart-type fatty acid-binding protein in the early diagnosis of acute myocardial infarction: a systematic review and meta-analysis. Heart. 2010 Nov 18;96(24):1957–63.

120. DeMaria EJ, Kenney PR, Merriam MA, Casanova LA, Gann DS. Survival after trauma in geriatric patients. Annals of Surgery. 2006 Jun 19;206(6):738–43.

121. Thacker SA, Robinson P, Abel A, Tweardy DJ. Modulation of the Unfolded Protein Response During Hepatocyte and Cardiomyocyte Apoptosis In Trauma/Hemorrhagic Shock. Sci Rep. 2013 Feb 1;3.

122. Zhang X, Lu C, Gao M, Cao X, Ha T, Kalbfleisch JH, et al. Toll-Like Receptor 4 Plays a Central Role in Cardiac Dysfunction During Trauma Hemorrhage Shock. Shock. 2014 Jul;42(1):31–7.

123. Azzazy HME. Unbound Free Fatty Acids and Heart-Type Fatty Acid-Binding Protein: Diagnostic Assays and Clinical Applications. Clinical Chemistry. 2006 Jan 1;52(1):19–29.

124. Kher A, Wang M, Tsai BM, Pitcher JM, Greenbaum ES, Nagy RD, et al. Sex Differences in the Myocardial Inflammatory Response to Acute Injury. Shock. 2005 Jan;23(1):1–10.

125. Ismailov RM, Ness RB, Weiss HB, Lawrence BA, Miller TR. Trauma associated with acute myocardial infarction in a multi-state hospitalized population. International Journal of Cardiology. 2005 Nov;105(2):141–6.

126. Krishna K Kadappu MBBS MD, Liza Thomas MBBS P. Tissue Doppler Imaging in Echocardiography: Value and Limitations. Heart, Lung and Circulation. Australian and New Zealand Society of Cardiac and Thoracic Surgeons (ANZSCTS) and the Cardiac Society of Australia and New Zealand (CSANZ); 2015 Mar 1;24(3):224–33.

127. Weng L, Liu Y-T, Du B, Zhou J-F, Guo X-X, Peng J-M, et al. The prognostic value of left ventricular systolic function measured by tissue Doppler imaging in septic shock. Crit Care. 2012;16(3):R71.

128. De Geer L, Engvall J, Oscarsson A. Strain echocardiography in septic shock – a comparison with systolic and diastolic function parameters, cardiac biomarkers and outcome. Crit Care. 2015;19(1):122.

129. Jäättelä A, Alho A, Avikainen V, Karaharju E, Kataja J, Lahdensuu M, et al. Plasma catecholamines in severely injured patients: A prospective study on 45 patients with multiple injuries. Br J Surg. John Wiley & Sons, Ltd; 1975 Mar 1;62(3):177–81.

130. Neil-Dwyer G, Cruickshank JM, Doshi R. The Stress Response in Subarachnoid Haemorrhage and Head Injury. In: Pickard JD, Cohadon F, Antunes JL, editors. Neuroendocrinological Aspects of Neurosurgery: Proceedings of the Third Advanced Seminar in Neurosurgical Research Venice, April 30–May 1, 1987. Vienna: Springer Vienna; 1990. pp. 102–10. (Neuroendocrinological Aspects of Neurosurgery: Proceedings of the Third Advanced Seminar in Neurosurgical Research Venice, April 30–May 1, 1987).

131. Norbury WB, Herndon DN, Branski LK, Chinkes DL, Jeschke MG. Urinary Cortisol and Catecholamine Excretion after Burn Injury in Children. The Journal of Clinical Endocrinology and Metabolism. The Endocrine Society; 2008 Apr 1;93(4):1270–5.

132. Opie LH. Heart Physiology: From Cell to Circulation. Lippincott Williams & Wilkins; 2004.

133. Hüttemann E, Schelenz C, Chatzinikolaou K, Reinhart K. Left ventricular dysfunction in lethal severe brain injury: impact of transesophageal echocardiography on patient management. Intensive Care Med. 2014 Apr 11;28(8):1084–8.

134. Prathep S, Sharma D, Hallman M, Joffe A, Krishnamoorthy V, Mackensen GB, et al. Preliminary Report on Cardiac Dysfunction After Isolated Traumatic Brain Injury*. Critical Care Medicine. 2014 Jan;42(1):142–7.

135. Hasanin A, Kamal A, Amin S, Zakaria D, Sayed El R, Mahmoud K, et al. Incidence and outcome of cardiac injury in patients with severe head trauma. Scand J Trauma Resusc Emerg Med. Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine; 2016 Apr 26;:1–6.

136. Chen Z, Tang L, Xu X, Wei X, Wen L, Xie Q. Journal of Critical Care. Journal of Critical Care. Elsevier Inc; 2017 Oct 1;41:240–6.

137. Mohseni S, Talving P, Wallin G, Ljungqvist O, Riddez L. Preinjury β-blockade is protective in

isolated severe traumatic brain injury. Journal of Trauma and Acute Care Surgery. 2014 Mar;76(3):804–8.

138. Schroeppel TJ, Fischer PE, Zarzaur BL, Magnotti LJ, Clement LP, Fabian TC, et al. Beta-Adrenergic Blockade and Traumatic Brain Injury: Protective? The Journal of Trauma: Injury, Infection, and Critical Care. 2010 Oct;69(4):776–82.

139. Johansson PI, Stensballe J, Rasmussen LS, Ostrowski SR. High circulating adrenaline levels at admission predict increased mortality after trauma. The Journal of Trauma: Injury, Infection, and Critical Care. 2012 Feb;72(2):428–36.

140. Ostrowski SR, Henriksen HH, Stensballe J, Gybel-Brask M, Cardenas JC, Baer LA, et al. Sympathoadrenal activation and endotheliopathy are drivers of hypocoagulability and hyperfibrinolysis in trauma. Journal of Trauma and Acute Care Surgery. 2017 Feb;82(2):293–301.

141. Seguin P, Laviolle B, Maurice A, Leclercq C, Mall dant Y. Atrial fibrillation in trauma patients requiring intensive care. Intensive Care Med. 2006 Jan 27;32(3):398–404.

142. Spahn DR, Bouillon B, Cerny V, Coats TJ, Duranteau J, Fernández-Mondéjar E, et al. Management of bleeding and coagulopathyfollowing major trauma: an updated Europeanguideline. Crit Care. BioMed Central Ltd; 2013 Apr 19;17(2):R76.

143. Kannel WB. Blood Pressure as a Cardiovascular Risk Factor. JAMA. 1996 May 22;275(20):1571.

144. Kannel WB, Abbott RD, Savage DD, McNamara PM. Coronary heart disease and atrial fibrillation: The Framingham Study. American Heart Journal. 1983;106(2):389–96.

145. Harrison DG, Florentine MS, Brooks LA, Cooper SM, Marcus ML. The effect of hypertension and left ventricular hypertrophy on the lower range of coronary autoregulation. Circulation. 1988 May 1;77(5):1108–15.

146. Lord JM, Midwinter MJ, Chen Y-F, Belli A, Brohi K, Kovacs EJ, et al. SeriesSurgical trauma 1The systemic immune response to trauma: an overview of pathophysiology and treatment. The Lancet [Internet]. Elsevier Ltd; 2014 Oct 18;384(9952):1455–65. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0140673614606875

147. Naganathar S, De'Ath HD, Wall J, Brohi K. Admission biomarkers of trauma-induced secondary cardiac injury predict adverse cardiac events and are associated with plasma catecholamine levels. Journal of Trauma and Acute Care Surgery. 2015 Jul;79(1):71–7.

148. Wilson NM, Wall J, Naganathar V, Brohi K, De'Ath HD. Mechanisms Involved in Secondary Cardiac Dysfunction in Animal Models of Trauma and Hemorrhagic Shock. Shock. 2017 Oct;48(4):401–10.

149. Jennings RB, Ganote CE. Structural changes in myocardium during acute ischemia. Circulation Research. 1974 Sep;35 Suppl 3:156–72.

150. Schaper J. Ultrastructural changes of the myocardium in regional ischaemia and infarction. European Heart Journal. 1986 Jul;7 Suppl B:3–9.

151. Jennings RB, BAUM JH, HERDSON PB. FINE STRUCTURAL CHANGES IN MYOCARDIAL ISCHEMIC INJURY. Arch Pathol. 1965 Feb;79:135–43.

152. Zong W-X, Thompson C. Necrotic Death as a Cell Fate. Genes and Development. 2005 Dec 19;(20):1–15.

153. Kemp M. Biochemical markers of myocardial injury. British Journal of Anaesthesia. 2004 Jul 1;93(1):63–73.

154. Otera H, Ohsakaya S, Nagaura Z-I, Ishihara N, Mihara K. Export of mitochondrial AIF in response to proapoptotic stimuli depends on processing at the intermembrane space. The EMBO Journal. 2005 Apr 6;24(7):1375–86.

155. Kloner RA, Ganote CE, Jennings RB. The "No-Reflow" Phenomenon after Temporary Coronary Occlusion in the Dog. J Clin Invest. 1974 Dec 1;54(6):1496–508.

156. Herdson PB, Sommers HM, Jennings RB. A Comparative Study of the Fine Structure of Normal and Ischemic Dog Myocardium with Special Reference to Early Changes Following Temporary Occlusion of a Coronary Artery. The American Journal of Pathology. 1965 Mar 1;46(3):367–86.

157. Jennings RB, Sommers HM, Herdson PB, Kaltenbach JP. ISCHEMIC INJURY OF MYOCARDIUM*. Annals of the New York Academy of Sciences. Blackwell Publishing Ltd; 1969 Jan 1;156(1):61–78.

158. Buja LM, Parkey RW, Stokely EM, Bonte FJ, Willerson JT. Pathophysiology of technetium-99m stannous pyrophosphate and thallium-201 scintigraphy of acute anterior myocardial infarcts in dogs. J Clin Invest. 1976 Jun 1;57(6):1508–22.

159. Shirakawa M, Imura H, Nitta T. Propofol protects the immature rabbit heart against ischemia and reperfusion injury: impact on functional recovery and histopathological changes. Biomed Res Int. 2014;2014:601250.

160. Martin M, Mullenix P, Rhee P, Belzberg H, Demetriades D, Salim A. Troponin Increases in the Critically Injured Patient: Mechanical Trauma or Physiologic Stress? The Journal of Trauma: Injury, Infection, and Critical Care. 2005 Nov;:1086–91.

161. Frobert A, Valentin J, Magnin J-L, Riedo E, Cook S, Giraud M-N. Prognostic Value of Troponin I for Infarct Size to Improve Preclinical Myocardial Infarction Small Animal Models. Front Physiol. 2015 Nov 27;6:1982.

162. Engle SK, Jordan WH, Pritt ML, Chiang AY, Davis MA, Zimmermann JL, et al. Qualification of Cardiac Troponin I Concentration in Mouse Serum Using Isoproterenol and Implementation in Pharmacology Studies to Accelerate Drug Development. Toxicologic Pathology. 2009 Jul 28;37(5):617–28.

163. Tao L. Mechanical traumatic injury without circulatory shock causes cardiomyocyte apoptosis: role of reactive nitrogen and reactive oxygen species. AJP: Heart and Circulatory Physiology. 2005 Jun 1;288(6):H2811–8.

164. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature. Nature Publishing Group; 2010 Apr 3;464(7285):104–7.

165. Cruickshank JM. Clinical importance of coronary perfusion pressure in the hypertensive patient with left ventricular hypertrophy. Cardiology. 1992;81(4-5):283–90.

166. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proceedings of the National Academy of Sciences. 2013 Feb 26;110(9):3507–12.

167. Sevrioukova IF. Apoptosis-Inducing Factor: Structure, Function, and Redox Regulation. Antioxidants & Redox Signaling. 140 Huguenot Street, 3rd FloorNew Rochelle, NY

10801USA: Mary Ann Liebert, Inc; 2011 Jun 15;14(12):2545–79.

168. Cabrera CP, Manson J, Shepherd JM, Torrance HD, Watson D, Longhi MP, et al. Signatures of inflammation and impending multiple organ dysfunction in the hyperacute phase of trauma: A prospective cohort study. Schreiber M, editor. PLoS Med. 2017 Jul 17;14(7):e1002352.

169. Waseem M, Tabassum H, Parvez S. Melatonin modulates permeability transition pore and 5-hydroxydecanoate induced KATP channel inhibition in isolated brain mitochondria. Mitochondrion. 31 IS -:1–8.

170. Kurdi MS, Patel T. The role of melatonin in anaesthesia and critical care. Indian Journal of Anaesthesia. India: Medknow Publications & Media Pvt Ltd; 57(2):137–44.

171. Hibaoui Y, Roulet E, Ruegg UT. Melatonin prevents oxidative stress-mediated mitochondrial permeability transition and death in skeletal muscle cells. J Pineal Res. Blackwell Publishing Ltd; 2009 Oct 1;47(3):238–52.

172. Gögenur I, Kücükakin B, Panduro Jensen L, Reiter RJ, Rosenberg J. Melatonin reduces cardiac morbidity and markers of myocardial ischemia after elective abdominal aortic aneurism repair: a randomized, placebo-controlled, clinical trial. J Pineal Res. 2014 Apr 25;57(1):10–5.

Appendix

Appendix I

Cambridgeshire 3 Research Ethics Committee
Victoria House
Capital Park
FULBOURN
Cambridge
CB21 5XB

Telephone: 01223 597597
Facsimile: 01223 597645

03 September 2010

Professor Karim Brohi
Consultant in Trauma, Vascular & Critical Care Surgery
Royal London Hospital
Trauma Clinical Academic Unit
Royal London Hospital
Whitechapel Road, London
E1 1BB

Dear Professor Brohi

Study Title:	**Trauma Associated Cardiac Injury, Dysfunction & Death (TACIDD)**
REC reference number:	**10/H0306/47**

Thank you for your letter of 23 August 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Mental Capacity Act 2005

I confirm that the committee has approved this research project for the purposes of the Mental Capacity Act 2005. The committee is satisfied that the requirements of section 31 of the Act will be met in relation to research carried out as part of this project on, or in relation to, a person who lacks capacity to consent to taking part in the project.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).
The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The

favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Investigator CV: Karim Brohi		07 June 2010
Protocol	1.1	02 June 2010
REC application	3	03 June 2010
Covering Letter		04 June 2010
Study Flowchart		07 June 2010
Letter from Sponsor		01 June 2010
GP/Consultant Information Sheets	1.1	02 June 2010
Participant Information Sheet: Information Sheet A - Subject	1.2	30 July 2010
Participant Consent Form: TACIDD A professional legally appointed representative consent form	1.2	30 July 2010
Response to Request for Further Information		23 August 2010
Participant Information Sheet: Information Sheet B - Subject	1.2	30 July 2010
Participant Information Sheet: Information Sheet C Consultee	1.2	30 July 2010
Participant Information Sheet: Information Sheet D - Consultee	1.2	30 July 2010
Participant Consent Form: A	1.1	02 June 2010
Participant Consent Form: Tissue	1.2	30 July 2010
Participant Consent Form: A -Subject	1.2	30 July 2010
Participant Consent Form: B -Subject	1.2	30 July 2010
Participant Consent Form: TACIDD B professional legally appointed representative consent form	1.2	30 July 2010
Evidence of insurance or indemnity: Barts & The London		01 June 2010

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

1. Notifying substantial amendments
2. Adding new sites and investigators
3. Progress and safety reports
4. Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

10/H0306/47	**Please quote this number on all correspondence**

Yours sincerely

Mr John Richardson
Chair

Email: lynda.mccormack@eoe.nhs.uk

Enclosures:	"After ethical review – guidance for researchers"
Copy to:	Mr Gerry Leonard R&D Department Queen Mary Innovation Centre Walden Street London E1 2EF

Appendix II

Barts and The London NHS
NHS Trust

Patient information sheet

DIRECTORATE OF SURGERY AND ANAESTHESIA
ROYAL LONDON HOSPITAL, WHITECHAPEL, LONDON E1 1BB

Information Sheet A – Subject
Version 1.2, 30/07/2010

Cambridgeshire 3 Research Ethics Committee
REC number: 10/H0306/47

Title: Trauma Associated Cardiac Injury and Dysfunction (TACID A)
Principal Investigator: Mr. Karim Brohi, FRCS FRCA

Date: ___/___/_____
Subject Name: _____. NHS Ref: _____ Study Ref: _____

Introduction

You are being invited to take part in a research study. This research will help us to improve the care of patients who suffer severe injuries in the future. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Why is this research being carried out?

Trauma (serious injury) is a serious health problem worldwide. Once discharged home, trauma patients are more likely to be limited in their daily activities compared to similar people who have not been injured. Injury, shock and blood loss may damage the heart, which might lead to the above. If this is the case, the extent and frequency of heart damage and dysfunction in trauma patients is not known, and we hope that this research will help us to determine if, who and how significantly the heart might be affected by trauma.

Coupled with this, we want to find out what happens to trauma patients who already have diseased hearts before injury, and compare their outcomes to similar patients with normal healthy hearts.

Why have I been chosen?

On ___ - ___ - _____ (date), you were injured and admitted to the Royal London Hospital. At the time, you were unable to give informed consent. When you arrived in the emergency department, a full trauma team of doctors and nurses attended to you. The trauma team leader, who is not part of this research study, gave consent as your representative. As part of the immediate management, a heart trace (ECG) was performed and blood taken and sent to the laboratory for analysis. A small amount of extra blood (approximately 1 teaspoonful) was drawn and saved for research purposes. We are now asking for your consent to allow us to use the samples we have collected and to continue to participate in the study, since all the procedures have not yet been completed.

Do I have to take part?

No, participation is completely voluntary. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

If you agree to continue with the study the following will happen:

1. We will store and process the samples we have already collected.
2. We will continue to collect blood samples and heart traces (ECGs) until the 3rd day in hospital. 3 of each in total. Each blood sample is equivalent to 1 teaspoonful, and the total amount of blood drawn over 3 days is less than 1 fluid ounce. Wherever possible we will draw the blood out of a line already in a blood vessel, or coincide the blood draw with tests required for your care, in order to minimise any discomfort from the procedure.
3. We will perform an echocardiogram (Echo) during your admission, at a time that is convenient to you and medically appropriate. An Echo is an ultrasound of the heart, and will give us information on the appearance and function of your heart.
4. This will take approximately 30 minutes, and is undertaking by placing some jelly on your chest followed by a probe that produces the images. It is very safe, but if the chest wall is injured, can be a little uncomfortable.

We will give you painkillers if this is the case, which will allow the test to be performed pain free.

5. When you go home, or at 30 days after your accident, we will ask you to fill in a questionnaire designed to assess your health perception. It is easy to complete and should take no more than 10 minutes.
6. We also need permission to access your health records, so that we can assess your health one year after your accident. This information is confidential, and will only be seen by the research team. It will only be used for the purpose described above in this study.

What do I have to do?

If you agree to continue with the study the following will happen:

1. We will collect ___ (number) of further blood samples and ___ ECGS from you, on _____(date/times)
2. We will perform an Echo at a convenient time to you during the course of your admission.

What are the possible disadvantages and risks of taking part in the study?

There are no long-term risks to you from participating in this study. The specific risks associated with each investigation are as follows:

1. Blood samples:
   The risks of drawing blood include temporary discomfort from the needle stick and bruising.
2. Echocardiogram:
   There is no risk to your health from an Echo, however, if you have chest wall injuries, you may find the procedure a little uncomfortable. We will give you painkillers if this is the case and only proceed when you are happy for us to do so.

What are the possible benefits of taking part in the study?

In the event that we detect an abnormality with your heart, we will refer you with your consent to a specialist in order to ensure you get appropriate care and follow up. In addition, we hope that the information we get will help to improve the care of trauma patients in the future.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

This Completes Part I.

If the information in Part 1 has satisfied you and you are considering continuing in the study, please read the additional information in Part 2 before making any decision.

Date: ___/___/_____ Researcher Initial: _____

PART 2

What will happen if I don't want to carry on with the study?

If you decide, at any time, to withdraw from the study all study procedures will be stopped immediately. Any information and samples that have already been collected will be processed as part of the study unless you wish to have your samples withdrawn from the study, in which case we will destroy them. Your decision will in no way result in a change in the type or quality of care you subsequently receive.

What if I am not happy about the study?

We will only make very minor changes to the way we look after you. It is extremely unlikely that this small change to normal practice would cause any problems. However, if taking part in this study harms you, there is no special compensation arrangement. If you are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay your legal costs. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you. Please contact Patient Advisory Liaison Service (PALS) if you have any concerns regarding the care you have received, or as an initial point of contact if you have a complaint. Please telephone 020 7377 6335, minicom 020 7943 1350, or email pals@bartsandthelondon.nhs.uk. You can also visit PALS by asking at any hospital reception.

Will my taking part in the study be kept confidential?

All information that is collected about you during the course of the research will be kept strictly confidential and will be stored securely in coded form. If you consent to take part in the research, the people conducting the study will abide by the Data Protection Act 1998, and the rights you have under this Act. Only authorised personnel such as researchers and research auditors will have access to the data. Any subsequent use of the samples will have to be performed with approval from a research ethics committee, otherwise the samples will be destroyed.

What will happen to the samples that I give?

We would like your permission to store your blood samples for further research. Any further use of your samples outside of this research study will have to be approved by a research ethics committee.

What will happen to the results of the research study?

We hope to publish the results in a scientific journal. It will not be possible to identify any individual who has taken part from this scientific report. Copies of the report will be available on request.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given ethical approval by the Cambridgeshire 3 Research Ethics Committee.

Who can I contact for further information?

1. If you require further information about the study, please contact the TACID study offices via the Trauma Surgery secretary at 020 7377 7000, x7695 or email: Henry.De'Ath@bartsandthelondon.nhs.uk
2. If you require impartial, local advice, please contact the Patient Advice and Liaison Service, telephone: 020 7943 1335 or e-mail: pals@bartsandthelondon.nhs.uk

Thank you for taking the time to read this sheet.

Date: ___/___/_____ Researcher Signature: _____

Appendix III

Information for Consultee

Barts and The London NHS
NHS Trust

DIRECTORATE OF SURGERY AND ANAESTHESIA
ROYAL LONDON HOSPITAL, WHITECHAPEL, LONDON E1 1BB

Information Sheet C - Consultee

Version 1.2, 30.07.2010

Cambridgeshire 3 Research Ethics Committee

REC number: 10/H0306/47

Title: Trauma Associated Cardiac Injury and Dysfunction (TACID A)

Principal Investigator: Mr. Karim Brohi, FRCS FRCA

Date: ___/___/_____
Subject Name: _____. NHS Ref: _____ Study Ref: _____

Introduction

We feel your relative/friend is unable to decide for himself/herself whether to participate in this research. To help decide if he/she should join the study, we would like to ask your opinion whether or not they would want to be involved. We would ask you to consider what you know of their wishes and feelings, and to consider their interests. Please let us know of any advance decisions they may make about participating in research. These should take precedence. If you are unsure about the role of consultee you may seek independent advice. We will understand if you do not want to take on this responsibility. The following information is the same as would have been provided to your relative/friend.

Why is this research being carried out?

Trauma (serious injury) is a serious health problem worldwide. Once discharged home, trauma patients are more likely to be limited in their daily activities compared to similar people who have not been injured. Injury, shock and blood loss may damage the heart, which might lead to the above. If this is the case, the extent and frequency of heart damage and dysfunction in trauma patients is not known, and we hope that this research will help us to determine if, who and how significantly the heart might be affected by trauma.

Coupled with this, we want to find out what happens to trauma patients who already have diseased hearts before injury, and compare their outcomes to similar patients with normal healthy hearts.

Why have they been chosen?

On ___ - ___ - _____ (Greenland P), your relative or significant other was injured and admitted to the Royal London Hospital. At the time, they were unable to give informed consent. When they arrived in the emergency department, a full trauma team of doctors and nurses attended to them. The trauma team leader, who is not part of this research study, gave consent as their representative. As part of the immediate management, a heart trace (ECG) was performed and blood taken and sent to the laboratory for analysis. A small amount of extra blood (approximately 1 teaspoonful) was drawn and saved for research purposes. We are now asking for your consent to allow us to use the samples we have collected and to allow their continued participation in the study, since all the procedures have not yet been completed.

Do I have to agree?

No, participation is completely voluntary. It is up to you to decide whether or not your relative/significant other should take part. If you do, you will be given this information sheet to keep and be asked to sign a declaration form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your relative/significant other receives.

What will happen to them if they take part?

1. If you agree to continue with the study the following will happen:
2. We will store and process the samples we have already collected.
3. We will continue to collect blood samples and heart traces (ECGs) until the 3rd day in hospital. 3 of each in total. Each blood sample is equivalent to 1 teaspoonful, and the total amount of blood drawn over 3 days is less than 1 fluid ounce. Wherever possible we will draw the blood out of a line already in a blood vessel, or coincide the blood draw with tests required for their care, in order to minimise any discomfort from the procedure.
4. We will perform an echocardiogram (Echo) during the admission, at a time that is convenient and medically appropriate. An Echo is an ultrasound of the heart, and will give us information on the appearance and function of the heart. This will take approximately 30 minutes, and is undertaking by placing some jelly on the patient's chest followed by a probe that produces the images.

It is very safe, but if the chest wall is injured, can be a little uncomfortable. We will give painkillers if this is the case, which will allow the test to be performed pain free.

5. When home, or at 30 days after the accident, we will ask your relative or significant other to fill in a questionnaire designed to assess their health perception. It is easy to complete and should take no more than 10 minutes.
6. We also need permission to access their health records, so that we can assess their health one year after your accident. This information is confidential, and will only be seen by the research team. It will only be used for the purpose described above in this study.

What do they have to do?

If you agree to continue with the study the following will happen:

1. We will collect ___ (number) of further blood samples and ___ ECGS from them, on_____(date/times)

2. We will perform an Echo at a convenient time to you during the course of his/her admission.

What are the possible disadvantages and risks of taking part in the study?

There are no long-term risks to you from participating in this study. The specific risks associated with each investigation are as follows:

1. Blood samples:

The risks of drawing blood include temporary discomfort from the needle stick and bruising.

2. Echocardiogram:

There is no risk to their health from an Echo, however, if they have chest wall injuries, they may find the procedure a little uncomfortable. We will give them painkillers if this is the case and only proceed when they are happy for us to do so.

What are the possible benefits of taking part in the study?

In the event that we detect an abnormality with your relative/significant other's heart, we will refer them with your consent to a specialist in order to ensure they get appropriate care and follow up. In addition, we hope that the information we get will help to improve the care of trauma patients in the future.

What if there is a problem?

Any complaint about the way you or your relative have been dealt with during the study or any possible harm they might suffer will be addressed. The detailed information on this is in Part 2.

Will taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about your relative/significant other will be handled in confidence. The details are included in Part 2.

This Completes Part I.

If the information in Part 1 has satisfied you and you are considering continuing in the study, please read the additional information in Part 2 before making any decision.

Date: ___/___/_____ Researcher Initial: _____

PART 2

What will happen if I don't want them to carry on with the study?

If you or your relative/significant other decides, at any time, to withdraw from the study all study procedures will be stopped immediately. Any information and samples that have already been collected will be processed as part of the study unless you wish to have their samples withdrawn from the study, in which case we will destroy them. Your decision will in no way result in a change in the type or quality of care they subsequently receive.

What if I am not happy about the study?

We will only make very minor changes to the way we look after your relative/significant other. It is extremely unlikely that this small change to normal practice would cause any problems. However, if they are harmed by taking part in this study, there is no special compensation arrangement. If they are harmed due to someone's negligence, then you may have grounds for legal action but you may have to pay your legal costs. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you or your relative have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you. Please contact Patient Advisory Liaison Service (PALS) if you have any concerns regarding the care they have received, or as an initial point of contact if you have a complaint. Please telephone 020 7377 6335, minicom 020 7943 1350, or email pals@bartsandthelondon.nhs.uk. You can also visit PALS by asking at any hospital reception.

Will taking part in the study be kept confidential?

All information collected during the course of the research will be kept strictly confidential and will be stored securely in coded form. If you consent to take part in the research the people conducting the study will abide by the Data Protection Act 1998, and the rights you and your relative have under this Act. Only authorised personnel such as researchers and research auditors will have access to the data. Any subsequent use of the samples will have to be performed with approval from a research ethics committee, otherwise the samples will be destroyed.

What will happen to the samples that are given?

We would like your permission to store his/her blood samples for further research. Any further use of the samples outside of this research study will have to be approved by a research ethics committee.

What will happen to the results of the research study?

We hope to publish the results in a scientific journal. It will not be possible to identify any individual who has taken part from this scientific report. Copies of the report will be available on request.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given ethical approval by the Cambridgeshire 3 Research Ethics Committee.

Who can I contact for further information?

1. If you require further information about the study, please contact the ACIT Study offices via the Trauma Surgery secretary at 020 7377 7000, ext7695 or email: Henry.De'Ath@bartsandthelondon.nhs.uk

2. If you require impartial, local advice, please contact the Patient Advice and Liaison Service, telephone: 020 7943 1335 or e-mail: pals@bartsandthelondon.nhs.uk

Thank you for taking the time to read this sheet.

Date: ___/___/_____ Researcher Signature: _____ _____

Appendix III

Trauma Associated Cardiac Injury and Dysfunction (A)

Trauma patients are eligible for participation in TACID A trial unless they meet one of the following:

- **Patients transferred from other hospitals**
- **Not expected to survive <72 hours**
- **Pregnant**
- **Patients <16**
- **Prisoners**
- **Trauma team leader deems recruitment inappropriate**

Patient Name _

Patient ID _ _ _ _ _ _ _ _ _ _ _ _

DOB _ _/_ _/__ Approximate Age _ _

Date Recruited _ _/_ _/_ _ Time of Baseline Blood/ECG _:_

I, as trauma team leader, fully understand the TACID A research study and informed consent process. I have read the consent documents and have provided consent in my capacity as a professional legally appointed representative of the above named patient and am independent of the trial.

Name _ _ _ _ _ _ _ _ _ _ _ _ _ _ Title _ _ _ _ _ _ _ _ _

Signature _ _ _ _ _ _ _ _ _ _ _ _ _

Appendix IV

Barts and The London NHS
NHS Trust

DIRECTORATE OF SURGERY AND ANAESTHESIA
ROYAL LONDON HOSPITAL, WHITECHAPEL, LONDON E1 1BB

Consent Form A – Subject

Version 1.2, 02.06.2010

Cambridgeshire 3 Research Ethics Committee

REC number: 10/H0306/47

Title: TRAUMA ASSOCIATED CARDIAC INJURY AND DYSFUNCTION (A)

Principal Investigator: Mr. Karim Brohi, FRCS FRCA

Please initial box to indicate agreement

1. I confirm that I have read and understood the information sheet dated 30.07.2010 (version 1.2) for the above study and have had the opportunity to ask questions. I have been given a copy of the patient's information sheet to keep. [ ]

2. I understand that my participation in this study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

3. I understand that sections of any of my medical notes may be looked at by professional individuals involved in this study or by regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. I understand that my personal data will be processed and stored securely in compliance with the 1998 Data Protection Act. [ ]

4. I agree to take part in the above study. [ ]

Name of patient	Date	Signature

I have explained this in terms which, in my judgement, are suited to the understanding of the patient.

Name of person taking consent (if different from Investigator)	Date	Signature

Investigator	Date	Signature

Appendix V

Barts and The London NHS
NHS Trust

DIRECTORATE OF SURGERY AND ANAESTHESIA
ROYAL LONDON HOSPITAL, WHITECHAPEL, LONDON E1 1BB

Consultee Declaration Form C

Version 1.2, 30.07.2010

Cambridgeshire 3 Research Ethics Committee

REC number: 10/H0306/47

Title: TRAUMA ASSOCIATED CARDIAC INJURY AND DYSFUNCTION (A)

Principal Investigator: Mr. Karim Brohi, FRCS FRCA

Please initial box to indicate agreement

1. I confirm that I have read and understood the information sheet dated 30.07.2010 (version 1.2) for the above study and have had the opportunity to ask questions. I have been given a copy of the consultee information sheet to keep. [ ]

2. I understand that his/her participation in this study is voluntary and that I am free to withdraw him/her at any time, without giving reason, without their medical care or legal rights being affected. [ ]

3. I understand that sections of any of his/her medical notes may be looked at by professional individuals involved in this study or by regulatory authorities where it is relevant to them taking part in research. I give permission for these individuals to have access to his/her records. I understand that his/her personal data will be processed and stored securely in compliance with the 1998 Data Protection Act. [ ]

4. I agree to allow my relative/significant other take part in the above study. [ ]

Name of Patient	Relationship	
Name of Consultee	Date	Signature

I have explained this in terms which, in my judgement, are suited to the understanding of the patient.

Name of person taking consent (if different from Investigator)	Date	Signature
Investigator	Date	Signature